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THE ASSAY OF PROGESTERONE BY THE PRODUCTION OF AN ARTIFICIAL PREGNANCY-RESPONSE OF THE FELINE UTERUS

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IT IS WELL known that Dale (1) and Cushny (2) independently discovered that the stimulation of the sympathetic fibers innervating the uterus of the cat produces relaxation of that organ in non-pregnant animals whereas a contraction is observed in pregnant animals. Van Dyke and Gustavson (3) were able to elicit a pregnancy-response (contraction) in ovariectomized non-pregnant cats by injecting a crude corpus luteum extract which probably contained considerable amounts of estrin. Estrin alone was without such action. Progesterone has since become available and was used by Kennard (4) who, however, employed large doses and did not first sensitize the uterus by injecting an estrogen. In a preliminary report, van Dyke (5) pointed out that progesterone (and hence progesterone-containing extracts) can be assayed by means of the induction of a pregnancy-response in normal or ovariectomized cats. The work here reported represents a detailed and rather extensive study of the conditions under which assay can be accomplished.

METHODS

The sequence of injections was standardized so that all observations, with the exception of histological examination of the uteri, could be completed within 7 days. The routine adopted for most of the experiment was as follows.

Day 1: 0.05 mg. (500 international units) of estradiol benzoate.
Day 2: 0.05 mg. (500 international units) of estradiol benzoate.
Day 3: No injection.
Day 4: One-third of the total dose of progesterone.
Day 5: One-third of the total dose of progesterone.
Day 6: One-third of the total dose of progesterone.
Day 7: Determination of the effect of intravenously injected adrenaline (and of hypogastric stimulation) on the uterine movements about 30 hours after the last injection of progesterone.

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When ovariectomized cats were used for assay, the operation was performed 4 to 10 days before injections were started.

The uterine response was determined under complete anesthesia by 'Evipal sodium'[2] which was injected intraperitoneally in a dose of 70 mg. per kg. body weight. Rarely this dose may cause considerable respiratory depression; therefore, a tracheal cannula was routinely inserted so that artificial respiration could be administered if necessary. The glass apparatus of figure 2 was adopted for recording uterine movements. The 'physiological' bath fluid containing phenol red and equilibrated to pH 7.4 by a mixture of 5% CO_2 in 95% oxygen was identical with that of van Dyke and Hastings

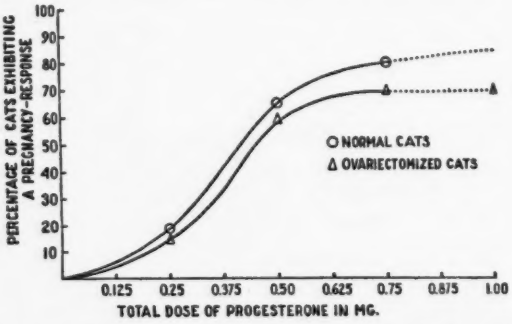


Fig. 1. The frequency of a pregnancy-response in normal and ovariectomized cats after the injection of various doses of progesterone. The sensitizing dose of estradiol benzoate was always 0.1 mg. The number of cats used for the determination of each point was not less than 20 except that represented by a dose of 1 mg. of progesterone in the ovariectomized cats. For this point 10 cats were used.

(6) except that the concentration of Ca^{++} was reduced to 0.75 mM per liter. It was siphoned from a thermostatically-controlled water bath to the inflow tube. The temperature at the outflow tube was maintained at 37° C. The uterus was exposed by a low, lateral incision of the abdominal wall and the medial part of the left uterine horn was tied to the blunt end of the inflow tube which could be disengaged from the rest of the apparatus. A string was also tied to the lateral end of the uterine horn for attachment, after being led over pulleys, to an isotonic lever magnifying movements about ten times. After the inflow tube had been replaced in the ground-glass joint, the whole apparatus was partly inserted in the abdominal cavity. The incised tissues of the abdominal wall were clamped tightly around the apparatus to prevent leakage and the flow of bath-fluid was started. For the determination of the effect of intravenously injected adrenaline or posterior-lobe extract this arrangement proved to be extremely satisfactory. However, when hypogastric stimulation was undertaken, it was usually necessary to reduce the flow of bath-fluid so that only moistening of the uterus was maintained. Otherwise there was too rapid an accumulation of the fluid in the exposed abdominal cavity.

[2] This drug is the sodium salt of 1,5-methyl-5-cyclohexenyl barbituric acid.

All the uteri were preserved for histological examination.

EXPERIMENTAL RESULTS

The results obtained when the injection procedure previously outlined was followed routinely are illustrated in figure 1 and are summarized in table 1. Ovariectomized cats appear to be less sensitive than normal cats. The total dose of progesterone necessary to alter the uterus so that a pregnancy-response appeared in 50% of the cats was estimated to be 0.41 mg. in normal cats and 0.45 mg. in ovariectomized cats. The discrepancy appears

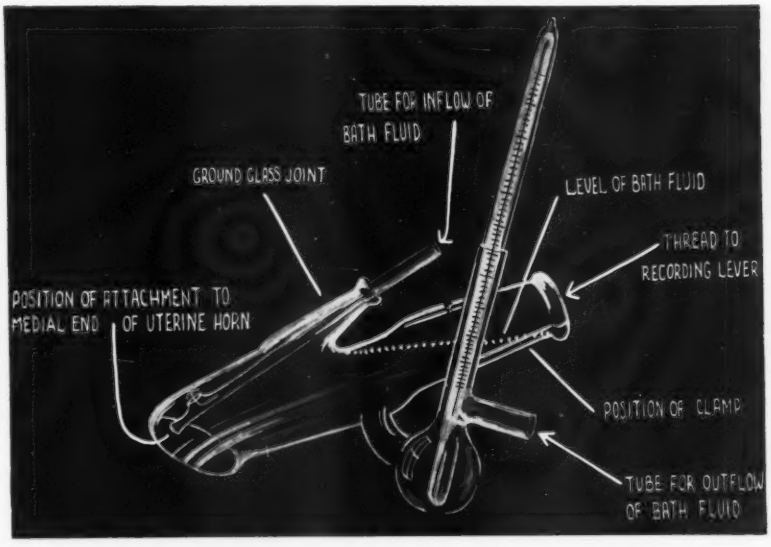

TUBE FOR INFLOW OF BATH FLUID
GROUND GLASS JOINT
LEVEL OF BATH FLUID
THREAD TO RECORDING LEVER
POSITION OF ATTACHMENT TO MEDIAL END OF UTERINE HORN
POSITION OF CLAMP
TUBE FOR OUTFLOW OF BATH FLUID

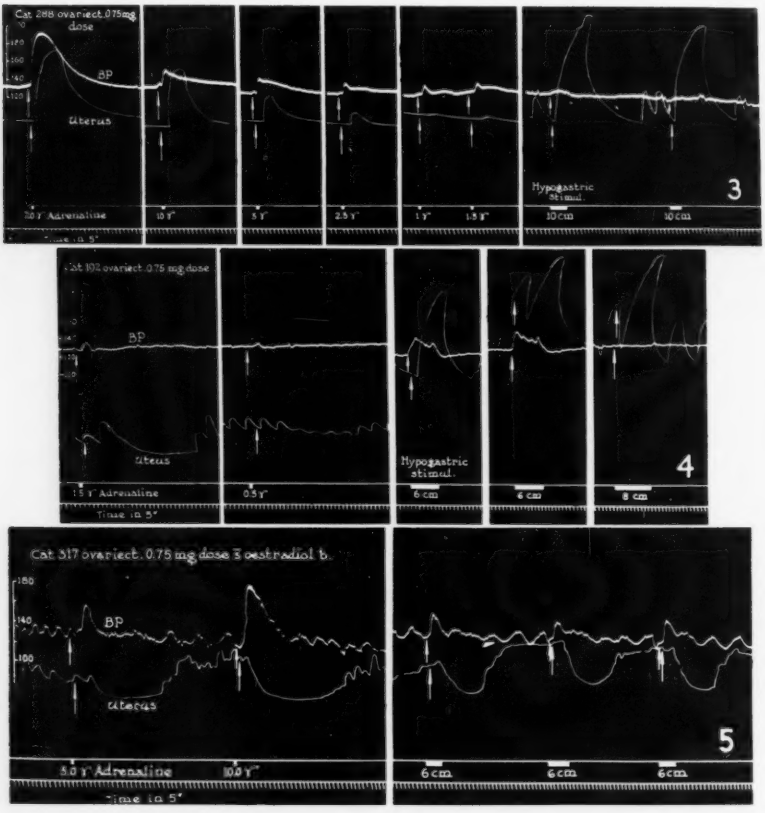
Fig. 2. Glass apparatus for recording contractions of the uterus in situ.

to be greater when the dose of progesterone is increased. For example, 0.54 mg. of the hormone was estimated to cause a pregnancy-reversal in 70% of normal cats whereas 0.75 mg. was required in ovariectomized cats.[3] The curves indicate that about 25% of cats respond poorly to the injection of estradiol benzoate and progesterone and that a relatively large dose of progesterone would be required to induce the phenomenon in all cats.

The data of figure 1 and table 1 refer to the response to intravenously injected adrenaline. The minimal dose required varied considerably; the average minimal dose in 100 cats was 2.5γ of adrenaline. In our experience contraction of the uterus following the intravenous administration of adrenaline is invariably associated with a contraction of the uterus accompanying hypogastric stimulation. On the other hand, in 13.5% of 74 cats in which hypogastric stimulation caused contraction, adrenaline induced only relaxa-

[3] In 10 ovariectomized cats the same frequency of pregnancy-reversal (70%) was observed after a total dose of 1.0 mg. of progesterone.

tion. This dissociation of action usually occurred after the injection of smaller doses of progesterone (0.25 to 0.50 mg.). The same phenomenon was previously reported by Kennard (4) who offered an explanation in terms of chemical mediators which might play different rôles. Diphasic responses such as



TRACINGS FROM EXPERIMENTS IN OVARIECTOMIZED CATS ALL RECEIVING 0.75 mg. OF PROGESTERONE. Fig. 3. Sensitizing dose of estradiol benzoate; 0.1 mg.; typical uterine contractions (positive response) followed the injection of adrenaline or hypogastric stimulation. Fig. 4. Estradiol benzoate as in 3. Adrenaline caused relaxation whereas hypogastric stimulation caused uterine contraction. Fig. 5. No estradiol benzoate administered. Relaxation of the uterus followed both adrenaline injection and hypogastric stimulation.

contraction followed by relaxation were also encountered. If the relaxation phase was slight and the contraction phase was prominent, a pregnancy-reversal was considered to be present. The reverse phenomena—a slight contraction phase and a pronounced relaxation phase—classified the response as a relaxation.

In the last part of table 1, results from control experiments are tabulated. The uteri of ovariectomized cats receiving no hormone whatsoever consist-

ently relaxed as a result of the injection of adrenaline or of hypograstric stimulation. The same result was obtained in normal cats receiving the usual sensitizing dose of estradiol benzoate (0.1 mg.). The importance of a 'priming' or sensitizing dose of an estrogen is believed also to have been demonstrated so far as the doses of progesterone used in our study are concerned. A dose of 0.75 mg. of progesterone in ovariectomized cats brought about a pregnancy-reversal in none of 10 cats, although, after a sensitizing dose of 0.1 mg. of estradiol benzoate, the same dose of progesterone caused the appearance of a pregnancy-reversal in 70% of the animals. If the dose of the estrogen was increased to 0.4 mg. a pregnancy-response likewise could be demonstrated in 70% of the cats. After the injection of 0.4 mg. of estradiol benzoate alone,[4]

TABLE 1. THE FREQUENCY OF A PREGNANCY-RESPONSE IN NON-PREGNANT NORMAL AND OVARIECTOMIZED CATS AFTER THE INJECTION OF ESTRADIOL OR PROGESTERONE OR BOTH

No. of cats	Ovariectomized	Total dose of		Pregnancy response	Remarks
		Estradiol benzoate	Progesterone		
		mg.	*mg.*	%	
21	No	0.10	0.25	19	
22	No	0.10	0.50	65	Estimated dose for 50% frequency=0.41 mg.
21	No	0.10	0.75	81	
5	No	0.10	1.00	80	
20	Yes	0.10	0.25	15	
22	Yes	0.10	0.50	59	Estimated dose for 50% frequency=0.45 mg.
20	Yes	0.10	0.75	70	
10	Yes	0.10	1.00	70	
10	Yes	0	0	0	
10	Yes	0	0.75	0	
10	No	0.10	0	0	
10	Yes	0.40	0	0	
10	Yes	0.40	0.75	70	

no pregnancy-response was elicited. However, in one cat of this group, all of which had been ovariectomized, a contraction was at first elicited; when uterine tone and movements gradually increased as the experiment progressed, no contraction phase was observed (fig. 9).

Other experiments, as outlined in table 2, were performed to determine whether pregnandiol or testosterone propionate produced any change in uterine response resembling that following the injection of progesterone. In the 4 experiments which could be performed with the amount of pregnandiol available, the uterus always behaved like a non-pregnant uterus although doses as high as 30 mg. were given. Testosterone propionate has been found to possess some pharmacological similarity to progesterone in that it may produce changes in the mucosa resembling those caused by progesterone. In 6 of our experiments a slight contraction preceded the predominant change, relaxation, following the injection of adrenaline or hypogastric stimulation. In the other two experiments only relaxation was observed. We con-

[4] The total dose was distributed over the same period as when 0.1 mg. estradiol benzoate and a dose of progesterone were given.

cluded that large doses of testosterone propionate do not induce a clear-cut pregnancy-reversal.

A few typical tracings are reproduced in figures 3–9. In the tracings of the uterine movements of 3 different cats, 0.75 mg. of progesterone had been administered to each (fig. 3–5). However, sensitizing doses of estrogen had been given to only 2 of the animals (fig. 3, 4). A typical pregnancy-response was observed in *cat 288* (fig. 3). In *cat 192* (fig. 4), only relaxation to adrenaline occurred. However, it appeared that hypogastric stimulation caused a contraction or an increase in contractility. The response was classified as

TABLE 2. THE EFFECT OF PREGNANDIOL OR TESTOSTERONE PROPIONATE ON THE RESPONSE OF THE CAT'S UTERUS TO ADRENALINE OR HYPOGASTRIC STIMULATION

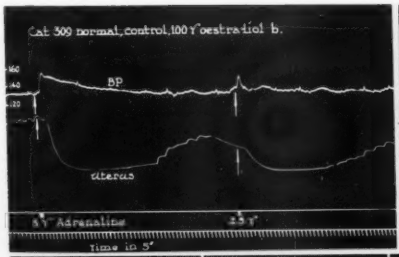
No. of cats	Ovariectomized	Total dose of			Remarks
		Estradiol benzoate	Pregnandiol	Testosterone propionate	
		mg.	*mg.*	*mg.*	
1	No	0.10	3.0		Relaxation
1	No	0.10	15.0		Relaxation
1	No	0.10	30.0		Relaxation
1	Yes	0.10	30.0		Relaxation
2	No	0.10		5.0	In these 6 cats a slight contraction preceded a marked relaxation
2	Yes	0.10		20.0	
1	Yes	0.10		22.0	
1	Yes	0.10		38.8	
1	Yes	0.10		10.0	Relaxation
1	Yes	0.10		20.0	Relaxation

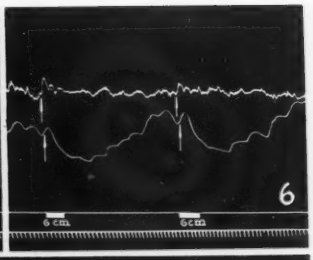
negative. *Cat 317* (fig. 5), received the same dose of progesterone without previous treatment with estradiol benzoate; this cat's uterus invariably relaxed, as was the case with the other 9 animals of this group. The remaining tracings illustrate the responses to adrenaline and hypogastric stimulation in cats receiving estradiol benzoate alone (fig. 6), pregnandiol (fig. 7), and testosterone propionate (fig. 8). Note that the uterus of the cat receiving testosterone propionate exhibited a minute contraction before relaxation and that this may have been related to the injection of adrenaline.

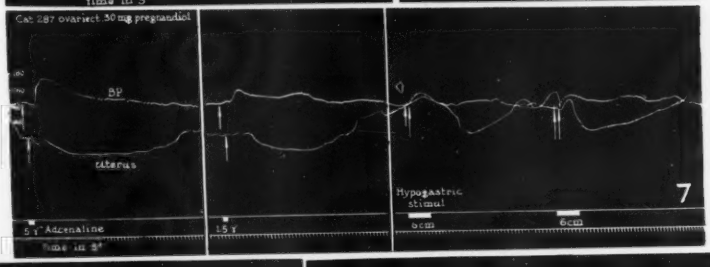
In one of the ovariectomized cats receiving 0.4 mg. of estradiol benzoate alone (fig. 9), 0.025 mg. of adrenaline or hypogastric stimulation caused contraction of the non-motile uterus early in the experiment. Uterine movements gradually became frequent and regular. Adrenaline or hypogastric stimulation then caused relaxation. This phenomenon was never observed in any of the other cats of this group.

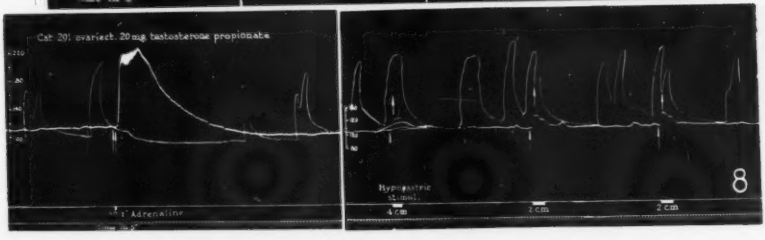
CONTROL EXPERIMENTS IN ALL OF WHICH THE UTERUS RELAXED AFTER EITHER TYPE OF STIMULATION. Fig. 6. This normal cat received the sensitizing dose of estradiol benzoate (0.1 mg.). Fig. 7 and 8. These ovariectomized cats both received 0.1 mg. of estradiol benzoate following which 30 mg. of pregnandiol (7) or 20 mg. of testosterone propionate were injected (8).

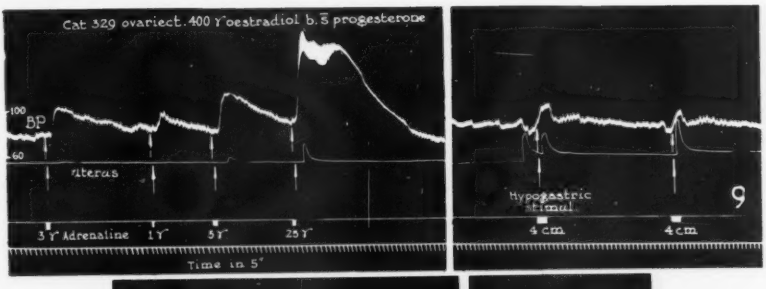
Fig. 9. THE ANOMALOUS BEHAVIOR OF THE UTERUS OF AN OVARIECTOMIZED CAT RECEIVING 0.4 mg. OF ESTRADIOL BENZOATE WITHOUT OTHER TREATMENT. (All the uteri of the other 9 cats of this group exhibited only relaxation in response to stimulation.) Early in the experiment, the non-motile uterus contracted after the injection of a large dose of adrenaline or hypogastric stimulation. Later, when spontaneous movements were prominent, stimulation produced only relaxation.

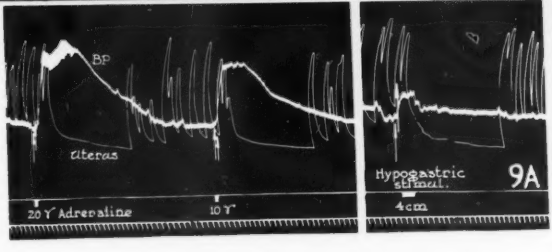

Cat 309 normal, control. 100 γ oestradiol b.
BP
uterus
5 γ Adrenaline
2.5 γ
Time in 5'


6 cm
6 cm
6

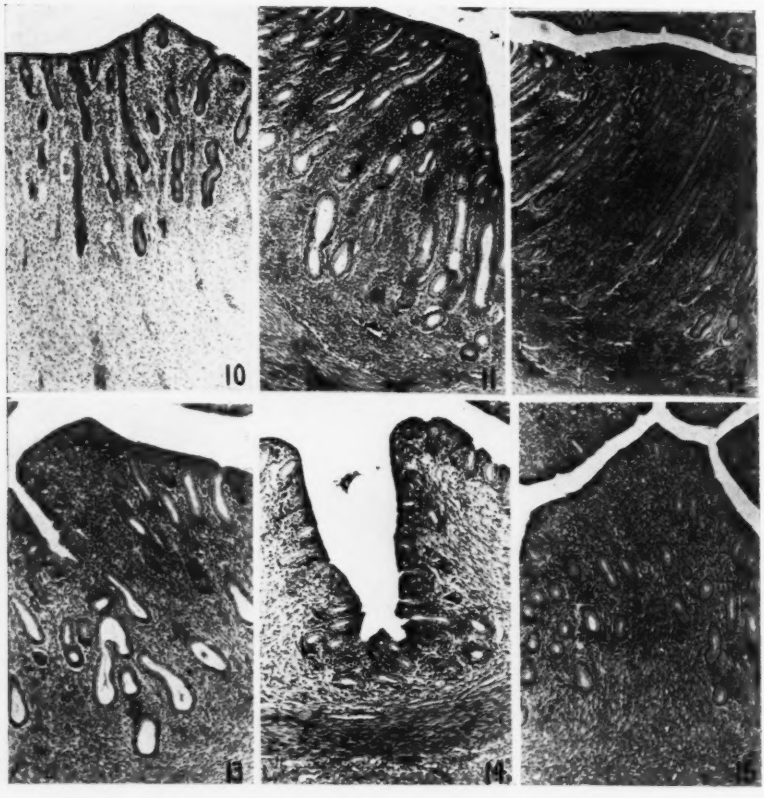

Cat 287 ovariect. 30 mg pregnandiol
BP
uterus
5 γ Adrenaline
1.5 γ
Hypogastric stimul.
6cm
6cm
7


Cat 201 ovariect. 20 mg testosterone propionate
Hypogastric stimul.
4 cm
2 cm
2 cm
8


Cat 329 ovariect. 400 γ oestradiol b. 5 progesterone
100
BP
60
uterus
3 γ Adrenaline
1 γ
5 γ
25 γ
Time in 5'
Hypogastric stimul.
4 cm
4 cm
9


BP
uterus
20 γ Adrenaline
10 γ
Hypogastric stimul.
4cm
9A

The approximately minimal effective intravenous dose of posterior-lobe extract also was routinely determined in nearly all the cats at the end of each experiment. There was considerable variation in the required dose. However, it was not possible to correlate this dose either with the presence of a preg-



PHOTOMICROGRAPHS, ALL AT THE SAME MAGNIFICATION, OF TYPICAL UTERI OF THE CATS USED. ALL THE CATS HAD BEEN OVARIECTOMIZED. Fig. 10. 0.1 mg. of estradiol benzoate followed by 0.5 mg. of progesterone. Stimulation caused contraction. Fig. 11. Same treatment as 10; however relaxation was the only response. Fig. 12–15. STIMULATION CAUSED ONLY RELAXATION OF THESE UTERI EXCEPT THAT UTERUS 14 EXHIBITED A SLIGHT CONTRACTION FOLLOWED BY A PRONOUNCED RELAXATION. TREATMENT WAS AS FOLLOWS. Fig. 12. 0.75 mg. of progesterone alone. Fig. 13. 0.4 mg. of estradiol benzoate alone. Fig. 14. 0.1 mg. of estradiol benzoate followed by 38.8 mg. of testosterone propionate. Fig. 15. 0.1 mg. of estradiol benzoate followed by 30 mg. of pregnandiol.

nancy-response or with the degree of growth and secretory activity of mucosal glands.

All the uteri were examined histologically. Photomicrographs of a few of the preparations are reproduced in figures 10–15. We were unable to establish any correlation between the response elicited and the degree of proliferation and secretion of the glands of the mucosa. This is illustrated by figures 10 and 11. Both cats were ovariectomized and received identical treat-

ment with estradiol benzoate and progesterone. It is doubtful that any change in the glandular epithelium of figure 10 is present; yet a pregnancy-response was consistently observed. On the other hand, there appears to be definite progestational proliferation of the glandular epithelium of figure 11; despite this change, the uterus always relaxed, behaving like that of a non-pregnant cat. All the other uteri illustrated by photomicrographs relaxed after chemical or electrical stimulation of the hypogastric nerves except that of the cat receiving testosterone propionate. In this cat a slight contraction was followed by a marked relaxation. It is clear that growth and secretory activity of the glands are much less in this cat (fig. 14) than in the two (fig. 12, 13) receiving progesterone alone or estradiol benzoate alone. The photomicrographs illustrate the difficulty of interpreting changes in the endometrial glands of the cat. The reader is also referred to the report of Rowlands and McPhail (7) who investigated the action of estrone and progestin on the anatomy of the cat's uterus.

DISCUSSION

The assay of progesterone by means of the frequency of appearance of a pregnancy-response is believed to possess both advantages and disadvantages in comparison with the commonly used histological methods based on changes in the uterus of the immature or adult rabbit. The chief advantage is that the reaction is usually definitely and unmistakenly either positive (contraction) or negative (relaxation). Moreover, the whole observation in at least a few cats can be completed within a week. The chief disadvantage of the method lies in the need for a crucial experiment in each cat to determine the response of the intact uterus. We are of the opinion that the use of the isolated cat's uterus, although much simpler technically, is less reliable. However, this can be determined only by more extensive experiments than the few preliminary observations we have made.

Also there can be no doubt that whether the method is based on histological changes in the immature or adult rabbit or on the pregnancy-reversal of the cat's uterus, it is desirable first to perform ovariectomy. Thus any effect of the sensitizing dose of estrogen on the formation or persistence of corpora lutea is avoided. All our observations were made in adult cats. It is possible that equally good results can be obtained in kittens in which the danger of any ovarian (corpus luteum) effect of the animal itself is as doubtful as in the immature rabbit.

For the routine experiments a large sensitizing dose of estrogen (0.1 mg. of estradiol benzoate) was chosen. Thus it was hoped that standardization of the uterine 'priming' could be accomplished with less variability than if a lower or a threshold dose had been employed. Presumably this would be true both of normal cats and of ovariectomized cats. In the former, natural variations in estrogen secretion occur, especially in relation to season. In the latter it often is not feasible to begin injections at fixed periods after ovariectomy.

The investigation of the threshold dose of posterior-lobe extract capable

of eliciting a uterine contraction was performed only after all the other observations had been made. It was concluded that the accuracy of observations made under these conditions is less than if this problem alone had been investigated. At any rate, no evidence of an altered response to the oxytocic action of posterior-lobe extract in relation to pregnancy-reversal or histological changes in the mucosa was observed.

SUMMARY

The assay of progesterone by producing an artificial pregnancy-response of the uterus of the cat (contraction in response to sympathetic stimulation) was studied. A preliminary sensitizing dose of estrogen is of great importance; for this purpose 0.1 mg. of estradiol benzoate was usually injected. The dose of progesterone causing a pregnancy-reversal in 50% of animals is estimated to be 0.41 mg. in normal adult cats and 0.45 mg. in ovariectomized adults. At higher dose levels, normal cats are relatively more responsive; however, it appears that the uteri of about 25% of all cats respond poorly. The above account refers only to the detection of the response by the intravenous injection of adrenaline.

Neither pregnandiol nor testosterone propionate causes a similar reversal in doses of 30 mg. or more.

The response of the feline uterus to sympathetic stimulation appears not to be related to the histology of the endometrial glands or to the sensitivity of the uterus toward the oxytocic action of posterior-pituitary extract.

Our thanks are due Prof. G. F. Marrian who kindly gave us a sample of pregnandiol of high purity. Schering-Kahlbaum A. G. of Berlin generously provided us with adequate amounts of solid estradiol benzoate and progesterone.

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EFFECT OF PROLACTIN ON MAMMARY GLAND SECRETION[1]

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PROLACTIN induces lactation in animals. Can an analogous action in women be inferred? Before testing the clinical value of prolactin, it is necessary to determine what constitutes normal milk secretion in women. Standards should be established to measure the apparent action of prolactin on human breast secretion. Three hundred and eighty consecutive cases have been studied with these problems in mind.

Normal and Deficient Breast Secretion

Authentic reports of measurements of normal human milk secretion are few. In 1909, Jaschke (1) reported the average daily milk consumption of 18 babies during the first 8 postpartum days. The birth weights varied between 2700 and 3410 gm. He included the average daily milk secretion as reported by 10 workers to which his own series was added (fig. 1). In the same year Rosenfeld (2) presented the daily intake of 21 babies during the first 7 postpartum days. The birth weights ranged from 2530 to 4100 gm. Alternate breasts were nursed every 3 hours. To determine the total daily secretion, both breasts were pumped 3 times daily, sometimes one hour before, at other times, one hour after the nursing period.

For our study, the nursing history and breast secretion of 380 consecutive nursing mothers were recorded. Records were limited to the first 10 postpartum days because of discharge from the hospital on the tenth day. On the basis of total milk secretion on the fifth postpartum day, the cases were classified as follows:

a), Full-breast nursing signifies an output greater than 250 cc. without complementary feedings being required.

b), Part-time nursing signifies an output greater than 250 cc. with complementary feedings being required.

c), Deficient nursing signifies an output less than 250 cc.

In our series of 380 cases, 333 mothers nursed their babies (table 1). Following the classification presented, 207 (61%) were full-breast nursing, 70 (21%) were part-time nursing and 56 (18%) deficient nursing.

The average daily milk consumption of 64 full-breast fed babies during the first 10 postpartum days is shown in figure 2. The curves show the greatest and least amounts consumed daily. The birth weights varied from 2680

[1] Read before the 22nd Annual Meeting of The Association for the Study of Internal Secretions, Saint Louis, Mo., on May 13, 1939.

to 4000 gm. They nursed alternate breasts every 4 hours beginning 12 hours after delivery. The babies were weighed before and after each breast feeding. Water was administered during the first 48 hours when indicated. After this period necessary complementary feedings were used.

During the first 5 days, the average secretion increased to 83% of the amount obtained at the end of the 10-day period. The curve of normal breast

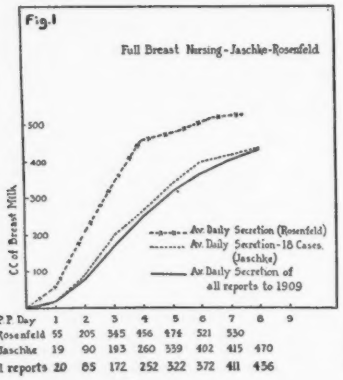
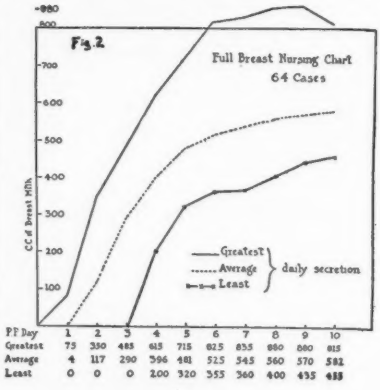


Fig. 1
Full Breast Nursing - Jaschke-Rosenfeld
CC of Breast Milk
Av. Daily Secretion (Rosenfeld)
Av. Daily Secretion - 18 Cases (Jaschke)
Av. Daily Secretion of all reports to 1909

P.P. Day	1	2	3	4	5	6	7	8	9
Rosenfeld	55	205	345	456	476	521	530		
Jaschke	19	90	193	260	359	402	415	470	
Av. All reports	20	85	172	252	322	372	411	456	

Fig. 1



P.P. Day	1	2	3	4	5	6	7	8	9	10
Greatest	75	330	485	615	715	825	855	880	880	815
Average	4	117	290	396	481	525	545	560	570	582
Least	0	0	0	200	320	353	360	400	435	488

Fig. 2

secretion rises abruptly the first 5 days and slowly the next 5 days. The point at which the curve changes is relatively constant. It is possible in most instances, therefore, to predict on the fifth day whether nursing will be adequate or deficient.

No mother with less than 250 cc. on the fifth day was able to nurse her baby adequately during the next 5 days. Of 70 part-time nursing mothers, 28%, or 1 of every 3½ cases were discharged as full-breast nursing on the

TABLE 1. EFFECT OF PROLACTIN ON MAMMARY GLAND SECRETION

Total nursing cases studied	380	
Never on breast	47 (12%)	
Nursing cases	333 (88%)	
Full-breast nursing 5th day		207 (61%)
Part-time nursing 5th day		70 (21%)
Deficient nursing 5th day		56 (18%)

tenth day. The possibility of raising inadequate milk secretion to full-time nursing is much greater if the output on the fifth day is more than 250 cc. A true deficiency of lactation may be assumed, therefore, when the total milk production is less than 250 cc. on the fifth postpartum day.

METHOD

Among 56 deficient lactating mothers only 24 expressed their desire: *a*), to nurse the baby for at least 6 weeks. *b*), to receive injections in an attempt to improve the milk supply. *c*), to pump the breasts every 4 hours for a minimum period of 5 days (fifth to ninth postpartum days).

Beginning the fifth postpartum day with the 10:00 A.M. nursing period an accurate measurement was made of the total output of breast milk in each 24-hour period. In 10 deficiency cases the baby was removed from breast during the experimental period. Alternate breasts were pumped every 4 hours day and night. An electric pump was used for a period of 10 minutes at a negative pressure of 5 mm. mercury. The amounts from each pumping were accurately recorded and the milk administered to the baby by bottle. In 14 of the deficiency cases, the baby continued to nurse alternate breasts every 4 hours for 10 minutes. Immediately after nursing, the breast was pumped for 5 minutes, or longer if necessary to completely empty the breast. The babies were carefully weighed before and after each nursing. If a total 24-hour output of 250 cc. or less of breast milk was obtained on the fifth day, the case was included in the deficiency study. This amount was exceeded in 8 cases not included in the deficiency study. All were instances where the baby was not completely emptying the breast. The deficiency was apparent but not real.

Identical appearing ampoules containing 5 cc. of the solution to be injected were differentiated by the labels 68A and 60A. The contents of these ampoules remained unknown until the study was concluded. After the results were tabulated, we learned that ampoules 68A contained 1000 pigeon units (Riddle) of lactogenic hormone. Ampoules 60A were control solutions containing no hormone.[2] On the fifth day, a skin test in the form of an intradermal injection of 0.1 cc. was made on the volar surface of the forearm. If the patient showed no hypersensitivity, 2.5 cc. (500 pigeon units) of the solution were injected subcutaneously at 9:00 A.M., and at 9:00 P.M. on the sixth postpartum day and on each subsequent day throughout the minimum 4 days of observation. The total daily dose was 1000 pigeon units.

In addition to the deficiency cases, 10 part-time nursing mothers were studied. Their total milk secretion on the fifth postpartum day was greater than 300 cc. Beginning at 10:00 A.M., on the fifth day, the baby nursed alternate breasts every 4 hours. The breast was pumped after each nursing. No injections were administered.

RESULTS

Prolactin. The average daily milk secretion of 14 deficiency cases receiving prolactin is represented in figure 3. The average secretion of 184 cc. on the fifth postpartum day increased to 212 cc. the following day. There was a decrease in 7 of the 14 cases on the seventh day, the average output falling to 205 cc. From the seventh to the ninth day, there was a progressive increase to 258 cc. The average total gain during the entire period was 74 cc. In relation to the amount secreted on the fifth day, this represents a gain of 40%. Three cases showed less milk production on the ninth than on the fifth day. None of this group was discharged with full-breast nursing.

Controls. Ten cases receiving the injection without prolactin showed a gradual rise from an average breast secretion of 219 cc. on the fifth day to 311 cc. on the ninth day. The total average gain during this period was 92 cc. or 42%. Two cases produced less milk on the ninth than on the fifth day. No case in this group was discharged with full-breast nursing.

Parity. The average gain (fig. 4) of primipara and multipara was 88 cc. (42%) and 81 cc. (44%) respectively. Three of 8 multipara showed a total gain greater than 100 cc. while only 4 of 16 primipara showed a gain greater than 100 cc.

[2] Prolactin and control solutions were generously supplied by Parke, Davis & Co.

Pumping-suckling. In 10 cases in which the breasts were pumped every 4 hours and the baby did not nurse, the average gain was 71 cc. or 43% (fig. 5). In 14 cases in which the breasts were pumped immediately after the baby nursed, the average gain in milk secretion was 89 cc. or 39%.

Age. The average secretion on the fifth day of 14 cases under 30 years of age was 215 cc. The daily average secretion gradually increased to 325 cc. on the ninth day. This was an increase of 110 cc. or 51%. In 10 cases over 30 years of age, the average output on the fifth day was 176 cc. On the ninth day the average output was 227 cc. The increase was 51 cc. or 28%.

Nursing history. The nursing history of 380 consecutive cases included the previous nursing record of the patient, her sisters and mother. From this data an attempt was made to predict in each case whether the present nursing would be full-breast, part-time or deficient. We were especially interested in predicting probable deficiency cases. So many variables were encountered that our predictions were too erratic to be useful.

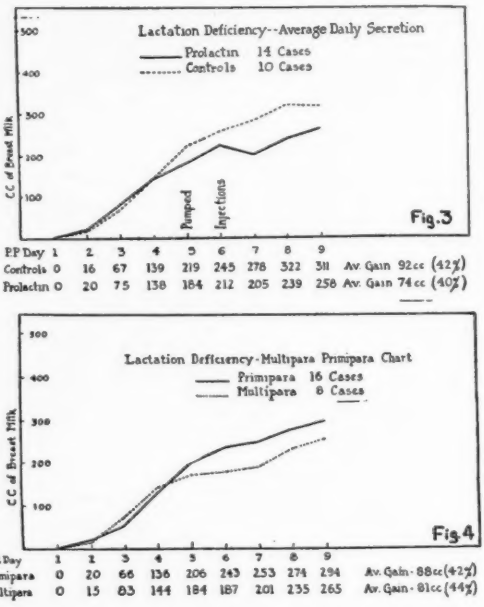

Lactation Deficiency--Average Daily Secretion
Prolactin 14 Cases
Controls 10 Cases
CC of Breast Milk
500
400
300
200
100
Pumped
Injections
Fig.3
P.P.Day 1 2 3 4 5 6 7 8 9
Controls 0 16 67 139 219 245 278 322 311 Av. Gain 92cc (42%)
Prolactin 0 20 75 138 184 212 205 239 258 Av. Gain 74cc (40%)
Lactation Deficiency-Multipara Primipara Chart
Primipara 16 Cases
Multipara 8 Cases
CC of Breast Milk
500
400
300
200
100
Fig.4
P.P.Day 1 2 3 4 5 6 7 8 9
Primipara 0 20 66 136 206 243 253 274 294 Av. Gain-88cc(42%)
Multipara 0 15 83 144 184 187 201 235 265 Av. Gain-81cc(44%)

Fig. 3 and Fig. 4

Follow-up. All 24 deficiency cases were seen at the end of the 6-week period. Ten mothers in the prolactin and control groups had dried up the breasts because of insufficient milk supply. Those who had received prolactin showed no significant increase of milk secretion over the control group.

Part-time. Part-time nursing mothers whose milk production on the fifth day was more than 300 cc. produced a greater average increase of milk secretion than the deficiency group (fig. 6). This series averaged 341 cc. on the fifth day and showed a progressive daily rise to 450 cc. on the ninth day. The total average increase of 109 cc. was greater than either the control or prolactin cases in the deficiency group. The percentage increase, however, was less (31%). Multipara showed a greater increase than primipara. The 2 cases in this group discharged with full-breast nursing were multipara.

DISCUSSION

The amount of milk consumed by the baby is not an accurate index of the

amount of milk the breasts are capable of secreting. Three cases of apparent lactation deficiency were pumped the fifth day and sufficient milk for full-breast nursing obtained. Many mothers had residual milk after the baby was satisfied. The total daily secretions were more than the greatest amounts consumed by the average breast-fed baby (fig. 2). One mother secreted sufficient milk to nourish 4 babies. These observations confirm the opinion of Macy and her coworkers (3) that complete and frequent emptying of the breasts encourages milk flow.

In general, there is a direct relation between the birth weight of the baby and the fifth day average milk consumption (fig. 7). There was a gradual rise of average milk consumption from babies weighing 2680 gm. to those weighing 4000 gm. Analysis of the individual feedings as well as the total daily feedings showed an individual variation of the amount of milk obtained by the baby at each nursing period as well as the total amount during each 24-hour period.

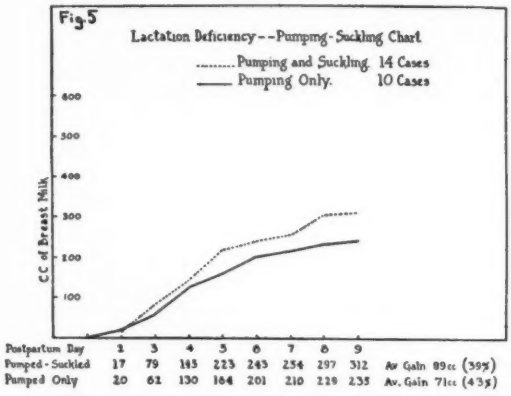


Fig. 5
Lactation Deficiency--Pumping-Suckling Chart
Pumping and Suckling 14 Cases
Pumping Only. 10 Cases
CC of Breast Milk
600
500
400
300
200
100
Postpartum Day 1 3 4 5 6 7 8 9
Pumped-Suckled 17 79 143 223 243 254 297 312 Av Gain 89cc (39%)
Pumped Only 20 61 130 184 201 210 229 235 Av. Gain 71cc (43%)

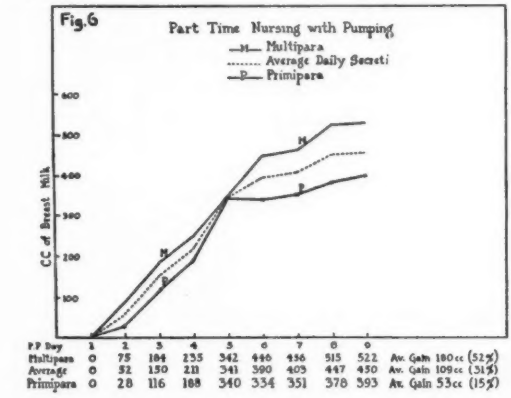


Fig. 6
Part Time Nursing with Pumping
—M— Multipara
Average Daily Secreti
—P— Primipara
CC of Breast Milk
600
500
400
300
200
100
PP Day 1 2 3 4 5 6 7 8 9
Multipara 0 75 184 235 342 446 436 515 522 Av. Gain 180cc (52%)
Average 0 52 150 211 341 390 403 447 450 Av. Gain 109cc (31%)
Primipara 0 28 116 188 340 334 351 378 393 Av. Gain 53cc (15%)

Fig. 5 and Fig. 6

In any clinical consideration of breast secretion, the age is an important factor. Comparison of age with the incidence of full-breast, part-time and deficient nursing is illustrated in figure 8. It was noted that 91% of mothers between the ages of 15 to 19 years were full-breast nursing. As the age increased the incidence of full-breast nursing decreased. Only 12% of those mothers aged 40 or over were classified as full-breast nursing. In contrast, the percentage of part-time and deficient nursing increased directly with age. In deficient nursing cases, the increase was from 9% in the 15 to 19-year group, to 50% in mothers 40 years or over.

Among the 24 deficient lactating mothers receiving prolactin or control solutions, there was a direct relationship between the age and average gain

in milk secretion. There was an average increase of 110 cc. or 51% in those cases under 30 years as compared with mothers over 30 years whose average increase was only 51 cc. or 28%.

Parity was apparently not an influencing factor in the deficiency group. In the 10 part-time nursing series whose breasts were pumped after each nursing period, multipara produced a greater average increase in breast milk than primipara. It is our impression that multipara showed a greater average increase of milk secretion than primipara. The contradictory results between multipara in the deficient and part-time study does not permit us to arrive at any definite conclusion.

Suckling did not alter deficient lactation. The average gain in the 10 cases where the baby was removed from breast was 71 cc. (43%). In 14 cases the baby continued to nurse but the average gain in milk secretion was 89 cc. (39%). This does not conform to the experimental results obtained in rats and mice by Selye (4), Selye, Collip and Thomson (5) and Selye and McKeown (6). They found that the suckling stimulus prevented involution for a period of 14 days even though no milk was removed from the lactating gland. On the basis of their work they believed that lactation was maintained by the suckling stimulus causing a discharge of prolactin from the pituitary gland. Reece and Turner (7) and Turner (8) state that

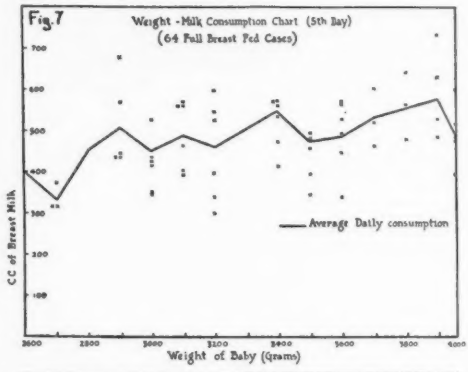
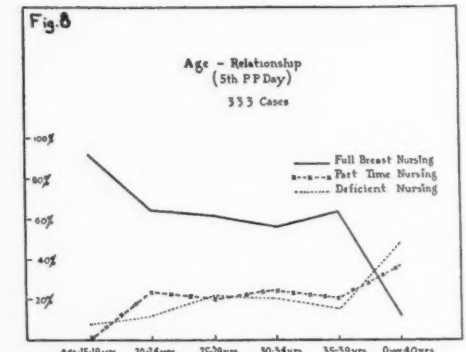


Fig.7
Weight - Milk Consumption Chart (5th Day)
(64 Full Breast Fed Cases)
CC of Breast Milk
Average Daily consumption
Weight of Baby (Grams)



	Age-15-19 yrs.	20-24 yrs.	25-29 yrs.	30-34 yrs	35-39 yrs.	Over 40 yrs
Full Nursing	10 (91%)	81 (65%)	57 (62%)	36 (50%)	22 (64%)	1 (12%)
Part Time Nursing	0	29 (23%)	16 (18%)	15 (25%)	7 (21%)	3 (38%)
Deficient Nursing	1 (9%)	14 (11%)	18 (20%)	14 (21%)	5 (15%)	4 (50%)

Fig. 7 and Fig. 8

The nervous stimulation of nursing either with or without the removal of milk caused a rapid discharge of the lactogenic hormone from the rat pituitary, presumably to stimulate the renewed secretion of milk. From these observations, it would appear that in some species, at least, the stimulus of nursing play a rôle in maintaining the secretion and discharge of lactogenic hormone, which in turn, maintains lactation.

Our experience with human breast secretion and the suckling stimulus supports the conclusion of Macy and her coworkers (3). They presented the

total daily quantity of milk produced by wet nurses during a period of 14 months. Their study included 2 successive lactation periods of one woman and a single period for two other subjects. They concluded: "lactation has been shown to persist at a high level over 14 months in the total absence of the suckling reflex."

The method of administration of prolactin presented disadvantages. Several mothers refused to undertake an attempt to increase breast secretion when they learned that injections would be necessary. Reactions to prolactin were discouraging. If skin tests are made before administering the drug, severe reactions may be avoided. Sensitivity tests were not attempted in two cases presenting manifestations of allergy. Four patients showing hypersensitivity to the intradermal injection were not used. Several cases were given injections although they demonstrated questionable sensitivity. Local reactions at the site of injections proved to be an unfavorable feature for routine administration. Local reactions became less frequent as the study progressed. No explanation can be offered for this change since bioassay revealed no loss of potency.

Selecting cases at random that have an apparent deficiency of lactation does not afford a good criterion of the effect of prolactin. Critical standards should be observed. Adequate controls are necessary. The age of the patient is important. Parity may influence the amount of milk secretion. A true deficiency must be determined for an apparent deficiency may develop to adequate nursing without therapy.

CONCLUSIONS

Weighing the baby before and after each nursing period and pumping the residual milk provides a satisfactory measure of breast secretion.

Whether the curve of secretion is high or low, the relative rise is greater during the first 5 postpartum days compared with the slow rise thereafter. The fifth postpartum day is the logical time to attempt to influence lactation. A true deficiency of lactation may be assumed if the daily secretion is 250 cc. or less by the fifth day.

Fourteen deficiency cases receiving 1000 pigeon units of prolactin daily from the sixth to the ninth postpartum day inclusively, showed a 40% increase in secretion. Ten deficiency cases as controls receiving injections of a similar fluid without prolactin showed a 42% increase in secretion. No influence on lactation could be attributed to prolactin. The action of prolactin in animals and women is not analogous. The suckling influence of the baby had no stimulating effect on deficient lactation.

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OBSERVATIONS ON THE ADRENALIN LEVEL IN THE BLOOD SERUM DURING INSULIN HYPOGLYCEMIA AND AFTER METRAZOL CONVULSIONS

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IN OUR STUDY on the effect of insulin shock therapy and the physical changes resulting from a severe hypoglycemia in the organism, the question arose as to the possible influence of the adrenal system on the clinical picture during the various stages of insulin shock. The interaction between insulin and adrenalin has been an established fact for a long time and "epinephrine may be considered as a direct antagonist of insulin in the truest sense because it attacks the same point in the carbohydrate cycle, exactly reversing the effect of insulin" (Peters and Van Slyke, 1). Britton and his coworkers (2) showed that animals deprived of the adrenal function were hypersusceptible to the hypoglycemic effect of insulin. Lately Crandall and Cherry (3) observed that adrenalectomized or adrenal denervated dogs went into shock as soon as one hour after the injection of 15 U of insulin. From their studies on the hepatic glucose output they concluded that the adrenal secretion is stimulated when the blood sugar level drops to a point between 50 and 60 mg.%. Brandt and Katz (4) demonstrated the presence of adrenalin in human blood during hypoglycemia induced by 20 to 30 U of insulin. They used the ear and intestines of rabbits and the eye of frogs as test organs. Riddle and coworkers (5) found the adrenal glands of their experimental animals to be enlarged in size and weight after insulin injections.

As yet, there has been no report in the literature regarding the adrenalin level in the blood during the various stages of insulin hypoglycemia. Such a longitudinal study would give a better understanding regarding the interaction of insulin and adrenalin and their rôle in the hypoglycemic syndrome. This paper deals with the findings of an investigation concerning the adrenalin content of the blood serum during the various phases of insulin hypoglycemia in schizophrenic patients.

METHOD

This study was undertaken with 25 patients. Ten cubic centimeters of blood was taken before treatment and following upon the injection of insulin similar quantities were removed at intervals of 30 minutes during the ensuing 3 to 4 hours and the last sample after the interruption of hypoglycemia upon the administration of sucrose. The serum was separated from the blood as soon as possible and mixed with 9 parts

of frog Ringer's solution. A Trendelenburg frog preparation was used for the test. In order to obtain a permanent record of the amount of fluid passing through the arteries in a definite period of time, the number of drops leaving the femoral artery was registered on a drum by allowing them to fall through the light beam of a photo-electric cell. In this way an electric circuit was closed each time a drop passed through the beam. The frog specimen was tested with frog Ringer's solution prior to each experiment until the amount of fluid passing through the arteries was constant over a period of at least 3 minutes. The amount of vaso-constrictor substances within the blood serum was measured by the difference between the number of drops of a normal frog Ringer's solution and the number of drops of the mixture of blood serum and Ringer's solution. In most cases whenever the amount of serum was sufficient, 3 determinations were made with each serum. Control experiments were carried out on the blood serum of schizophrenic patients not receiving any therapy.

RESULTS

The survey of our results shows that the material can be grouped into three distinct categories.

A. A group in which the patients went into shock readily and remained in coma until the hypoglycemia was interrupted.

B. A group in which the insulin administration did not seem to affect the patient clinically or in which only slight drowsiness and mild perspiration were noticeable.

C. A group in which the patient did not go into shock rapidly, but rather seemed to fluctuate between a wakeful state and deep coma. Most of the patients of this group displayed severe muscular twitchings and myoclonic movements.

In the patients of the first group the injection of insulin was followed by a slight rise of the adrenalin level of the blood, but in many cases there was a decrease in the amount of adrenalin after 60 minutes. After 90 minutes there was a marked decrease in the adrenalin content in all the cases when compared with the fasting level. This low level was maintained until the shock was interrupted by the administration of sucrose. At that time the adrenalin level started to rise very rapidly and remained high for the remainder of the observation period. This marked adrenalemia was simultaneous with the awakening of the patient (fig. 1).

In the patients of our second group with none or only mild reaction to insulin the level of the vasoconstrictor substances in the blood serum rose sharply 30 minutes after the injection. The amount of adrenalin was definitely higher 60 minutes after the administration of insulin as compared with the fasting level. Thereafter the amount of adrenalin continued to remain high and even increased within the next 30 minutes. In certain cases of this group a drop of the adrenalin level was noted 2½ hours after the injection. It seemed to us that this was an indication that the self-regulating mechanism of the organism had completed its counteraction to insulin (fig. 2).

The curves in our last group did not seem to follow any definite pattern. In these cases the amount of adrenalin seemed to depend more upon the muscular movements of the patient than upon the time which had elapsed

following injection. A definite relationship, however, was seen between the amount of adrenalin present in the blood serum and the depth of the hypoglycemia. The adrenalin content was always low in cases in which the blood was taken at the time the patient was in deep coma. However, in those cases with severe myoclonic twitchings a high adrenalin level was obtained when tested half an hour after these muscular movements had begun (fig. 3). Quite frequently these patients showed alternating periods of wakefulness and coma, correlated with heights and depressions of the adrenalin

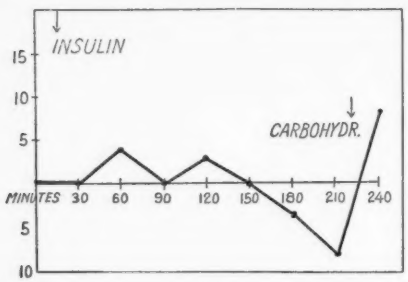

FIG. 1

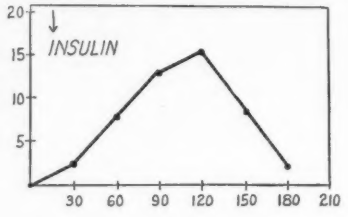

FIG. 2

Fig. 1. COMPOSITE CURVE OF ALL TYPICAL SHOCK PATIENTS. CONTINUOUS DIMINUTION OF VASOCONSTRICTOR SUBSTANCES IN THE SERUM. SHARP RISE AFTER ADMINISTRATION OF CARBOHYDRATES. (THE ABSCISSA VALUES ARE EXPRESSED AS THE DIFFERENCE FROM THE INITIAL VALUE.) Fig. 2. 'NON-SHOCK' CURVE. PATIENT *S. Qu.*, 60 U INSULIN. CONTINUOUS COMPENSATORY INCREASE OF VASOCONSTRICTOR SUBSTANCES. SELF-REGULATION COMPLETED AFTER 2 HOURS. (THE ABSCISSA VALUES ARE EXPRESSED AS THE DIFFERENCE FROM THE INITIAL VALUE.)

curve. Such a fluctuation between high and low levels of adrenalin reflecting the change in the hypoglycemic reaction of the patient occurred in some cases more than once a morning.

DISCUSSION

Our observations show that 30 to 60 minutes after the injection of insulin the adrenal apparatus tends to respond by raising the epinephrine level of the blood serum. The adrenalin secretion is less marked in the so-called coma cases than in those individuals who do not present any evidence of shock. One and one-half hours after the insulin injection a definite difference could be observed in the adrenalin level between the 'shock' and 'non-shock' cases. The patients of the first group showed a drop of the adrenalin content, while in those patients who showed less or no clinical symptoms, a decided rise occurred. This difference was not at all dependent upon the weight of the patient nor upon the amount of insulin given, since non-shock curves could be obtained with a dosage as low as 10 and as high as 300 U while the same was true of the shock patients.

A definite quantitative relationship, however, was noted in those individual patients who did not respond to a high dose of insulin, but did so after the dose was reduced by 40 or 50% the next day. They showed a non-shock curve to the high dosage of insulin, while the adrenal apparatus did not seem to respond to a considerably smaller amount of insulin. This led us to believe

that the insulin coma is directly proportional to the adrenal response in these cases. With a relatively large amount of insulin the adrenal apparatus is stimulated to such a degree that the hypoglycemic effect is compensated, while a comparatively low dosage of insulin does not produce a sufficient stimulus to activate the adrenal system to a compensating degree. Thus in the latter case the insulin exerts its full hypoglycemic effect on the blood sugar level and on the tissues so that the patient reacts with a deep coma. This hypothesis explains the fact that patients who very often fail to respond to a very high dosage of insulin react to a low one, and also gives some understanding of the sensitizing effect of insulin. Other factors, too, are involved in this mechanism, i.e., the amount of available glycogen within the liver and probably the activity of the anterior pituitary lobe.

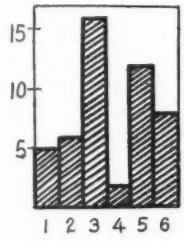

Fig. 3. Relationship between adrenalin level and clinical picture (25 cases). (The abscissa values represent the difference between test reaction of control and experimental solutions.) 1. Fasting level. 2. Beginning hypoglycemia. 3. Marked muscular activity. 4. Coma. 5. Self-regulation. 6. After feeding.

According to Cori (1), epinephrine acts on the carbohydrate metabolism by impairing or preventing the oxidation of carbohydrates. In patients presenting a non-shock curve, the amount of adrenalin in the blood stream is comparatively high since the adrenal apparatus counteracts or prevents the oxidation of glucose. In patients, who go into shock very readily, however, the amount of glucose in the blood stream falls to an almost zero level. Since glucose is no longer present in the blood, one cannot expect an adrenalin reaction at this stage. The adrenal apparatus responds as long as glycogen can be liberated from the liver, therefore the adrenal secretion will not depend only upon the amount of insulin given, but also on the availability of glycogen in the liver. As soon as glucose reaches the blood stream, as in the time of feeding, a marked response of the adrenal gland occurs again, preventing a storage of glycogen as long as there is a lack of carbohydrates within the tissues. Such a sequence of events was present in our shock cases.

In our third group of patients, who did not present a regular curve, the sequence observed in the first 2 groups seems to be overshadowed by the marked muscular twitchings and by the occurrence of myoclonic movements. It is known that patients very often awaken after muscular exertion, simultaneously the adrenalin level in the blood serum rises. This level very often exceeds that found in the patients of groups *A* and *B*. The adrenalin content seems to reflect directly the various stages of insulin hypoglycemia. The

level is high when the patient is awake or when he is recovering from the hypoglycemic state, while it reaches an almost zero level during coma (fig. 3).

Similar experiments were carried out before and during metrazol convulsions, and during varying periods thereafter in order to study the influence of severe myoclonic contractions on the adrenalin output. It was noted that the metrazol raised the adrenalin level even in the interval between injection and convulsion, but that it reached the highest level approximately 25 minutes after the convulsive period. The adrenalin level continued to be high for 2 hours and returned to normal after 2½ hours. The epinephrine content of the blood serum was negligible in patients who did not respond with a convulsive seizure, indicating that the response of the adrenal glands to metrazol was dependent on the convulsive action of the drug.

SUMMARY

The activity of adrenalin and adrenalin-like substances in the blood serum was studied in patients during various hypoglycemic states. Three different types of reactions were obtained. The curves of those patients evidencing shock showed an inadequate adrenal response to insulin 90 minutes after the injection, while the non-shock curves displayed a marked rise in the adrenalin level of the blood at this time. The adrenalin output in the third group was dependent upon the muscular movements; this was confirmed by observations on the adrenalin level blood serum after metrazol convulsions. The adrenalin level reflected directly the various stages of insulin hypoglycemia. At the time of deep coma the adrenalin level was very low, while it rose to a high level at the time of recovery.

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THE ESTROGEN-PROGESTERONE INDUCTION OF MATING RESPONSES IN THE SPAYED FEMALE RAT[1]

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ESTROGEN and progesterone administered in the proper sequence have been found to be more effective than estrogen alone in inducing sexual receptivity in the guinea pig (1). Since this was the only species in which this action of estrogen and progesterone had been observed, it seemed desirable to ascertain if these hormones acting synergistically are not also effective in inducing heat in another species such as the rat. The rat was chosen because the occurrence of mating in 'constant estrous animals' which ordinarily do not mate, ovulate, or form corpora lutea has been reported following the injection of luteinizing hormone (2, 3). The induced mating was associated with ovulation and corpus luteum formation and suggested a relationship between the appearance of the corpora lutea and mating, similar to that postulated for the guinea pig. The possibility has been investigated and data, which indicate that in this respect the rat is similar to the guinea pig, are given below.

EXPERIMENTAL

Thirty 3 to 7-month-old spayed rats of the Wistar strain were used. Estrogen (estradiol benzoate) and progesterone[2] in corn oil were injected subcutaneously. The animals were given a rest period of 10 or more days between each series of injections.

The procedure for all experiments was essentially as follows. The estrogen was administered in a single injection, with the exception of 10 animals which received 100 R.U. in 3 doses of 50, 25 and 25 R.U. over a period of 48 hours. Hourly observations for evidences of heat were begun 24 to 40 hours after injection of the estrogen and continued until the 48th, 72nd or 96th hour. Except as noted below the animals which did not come into heat on the estrogen received a single injection of 0.2, 0.3 or 0.4 I.U. progesterone. Hourly observations were then continued until the end of heat or, when heat did not occur, for 20 to 24 hours.

Two carefully graded systems have been proposed for classifying the widely varying intensities of heat responses observed during the normal cycle of the rat (4, 5). In the present work only two types of behavior were em-

[1] The investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council; grant administered by Prof. William C. Young.
[2] Progynon-B and proluton for which we are indebted to Dr. Erwin Schwenk of the Schering Corporation.

ployed in recording the response to hormonal action: first, that displayed by animals which did not respond immediately to the attention of the male, and second, that displayed by animals which responded immediately, even to less vigorous males, and gave a marked lordosis when stroked on the dorso-posterior region. The latter type of behavior is characteristic of that displayed by normal animals at the height of heat. A negative response was considered to have been obtained only when the female would not mate after repeated attempts of vigorous males.

TABLE 1. THE INDUCTION OF HEAT BY INJECTION OF ESTROGEN (ESTRADIOL BENZOATE)

No. of injections	Quantity of injected estrogen	Length of observation after estrogen injection	No. of injections followed by heat	Latent period	
				Average	Range
	R.U.	*hours*		*hours*	*hours*
44	5	48	0.0	—	—
6	5	72	0.0	—	—
38	10	72	6	52	48 to 54
19	10	96	7	56	<39 to 74
10	20	72	2	49.5	49 to 50
20	100	96	18	50	38 to 67

Induction of Heat by Estrogen Alone

Five R.U. of estradiol benzoate given in a single injection did not induce mating in any one of 44 animals observed for 48 hours, or in any one of 6 animals observed for 72 hours after the injection (table 1). The only indication of sexual activity was a quivering of the ears (4, 5) without mating which was observed in one animal beginning the 45th hour after injection.

The injection of 10 R.U. of estradiol benzoate was followed by heat in 6 of 38 animals observed 72 hours, and in 7 of 19 observed 96 hours. The latent period between injection and the beginning of heat averaged 52 hours in animals of the first group, with a range of 48 to 54 hours. In animals of the second group, the latent period between injection and the beginning of heat averaged 56 hours, and ranged from <39 to 74 hours. This group was established when it was found that in 2 animals the latent periods for estrogen-induced heat were 72 and 74 hours. The possibility existed, therefore, that had the first group of animals been observed longer than 72 hours, additional cases of estrogen-induced heat would have been found. Data which were obtained subsequently make this possibility seem unlikely. In the first place, in no other case among the animals in the second group did a latent period exceed 63 hours. Secondly, 20 animals were injected with 100 R.U. of estrogen and observed for 96 hours. In no one of the 18 which came into heat did the latent period exceed 67 hours.

In the case of the 2 groups of animals injected with 10 R.U. of estrogen, the length of heat varied from 1 to 7 hours. The intensity of the response was usually of a low grade; vigorous males were required to obtain a mating from most animals. Quivering of the ears was observed in the animals which came into heat and in some in which heat did not occur.

Two of 10 animals given single injections of 20 R.U. of estradiol benzoate mated after latent periods of 49 and 50 hours. One animal remained in heat 4.5 hours while the other mated intermittently for 21 hours. This intermittent mating was characteristic of heat induced by large injections of estrogens alone. The intensity of the mating responses was similar to that of responses observed after the injection of 10 R.U. of estrogen.

In order to determine what percentage of animals could be brought into heat by large injections of estrogen, 20 were injected with 100 R.U. of estradiol benzoate. Ten received the entire amount in one injection. The remaining 10 were given an initial injection of 50 R.U. followed by injections of 25 R.U., 24 and 48 hours later. Nine of the 10 animals receiving a single injection came into heat after latent periods which averaged 50 hours, range 46 to 67 hours. Similarly, 9 of 10 animals receiving 3 injections came into heat. The latent periods averaged 49 hours and ranged from 38 to 67 hours. In the first group of animals heat was displayed for an average of 19 hours (range 2 to 29 hours) although frequently it was intermittent. In the second group the period during which heat was displayed averaged 112 hours with a range of 3 hours to 10 days. In this group, also, especially during the daylight hours, long periods occurred when the females would not mate with even the most vigorous males. Furthermore, in both groups, when the animals would mate, the intensity of the responses varied greatly. Occasional animals showed a very intense heat, but generally the responses were not as intense as those displayed during a normal or an estrogen-progesterone induced heat.

Induction of Heat by Estrogen-Progesterone Injections

Twenty-two of the 44 animals injected with 5 R.U. estradiol benzoate and which did not come into heat following this treatment, received 0.4 I.U. of progesterone 48 hours later. In this group progesterone was injected on the 48th hour because other experiments had demonstrated that 5 R.U. of estrogen is not sufficient to keep all animals conditioned 72 hours. Twenty of the animals came into heat after a latent period which averaged 4.5 hours and ranged from 3 to 8 hours (table 2). The length of heat averaged 10.5 hours (range 2 to 14 hours). In general the response of these animals was not as intense as that observed in normal animals or in animals conditioned with larger quantities of estrogen. Vigorous males were needed for most matings.

Fifteen of the 44 animals injected with 10 R.U. estradiol benzoate which did not come into heat received 0.4 I.U. progesterone on the 72nd hour. All came into heat. The latent period between the progesterone injection and heat averaged 2.7 hours (range 1.5 to 4 hours). The length of heat averaged 11.5 hours (range 7.5 to 15.5 hours). The intensity of the response was comparable to that of normal animals. Lordosis could easily be elicited by non-aggressive males and in the absence of males the copulatory response was readily obtained by stroking the animals on the back.

When the animals which had failed to come into heat following the larger dosages of estrogen were injected with progesterone, the results were the same. Eight injected 72 hours previously with 20 R.U. of estrogen and 2 in-

jected 96 hours previously with 100 R.U. came into heat after receiving 0.4 I.U. progesterone. The latent period after progesterone injection and the length of heat were about the same as those recorded when animals were injected with 10 R.U. estrogen followed by progesterone (table 2).

TABLE 2. THE INDUCTION OF HEAT BY ESTROGEN-PROGESTERONE ACTION

No. of injections	Quantity of injected estrogen	Interval between injections	Quantity of injected progesterone	No. of injections followed by heat	Latent period		Length of heat	
					Average	Range	Average	Range
	R.U.	*hours*	I.U.		*hours*	*hours*	*hours*	*hours*
22	5	48	0.4	20	4.5	3–8	10.5	2–14
22	5	48	0.3	15	5.2	3–11	9.6	2.5–16
15	10	72	0.4	15	2.7	1.5–4	11.5	7.5–15
15	10	72	0.3	15	2.4	2–3	9.6	9–11
8	20	72	0.4	8	2	2–2	unknown	11 to 23
2	100	96	0.4	2	3	3–3	9.5	9 & 10
6	10	96	0.4	6	3	3–3	unknown	unknown

When the quantity of injected progesterone was reduced from 0.4 I.U. to 0.3 I.U. a slight decrease in effectiveness was noted. Fifteen of 22 animals previously injected with 5 R.U. of estrogen came into heat when 0.3 I.U. of progesterone was injected 48 hours later. All of 15 animals receiving 0.3 I.U. of progesterone after an initial injection of 10 R.U. of estrogen came into heat, but the response was not as intense as that of animals receiving 0.4 I.U. of progesterone after the same amount of estrogen. When the quantity of injected progesterone was reduced to 0.2 I.U., only 10 of 25 adequately conditioned animals came into heat and the possibility cannot be excluded that several of these were brought into heat by the action of the estrogen alone. It is apparent, therefore, that under the conditions of this experiment, 0.3 I.U. to 0.4 I.U. of progesterone is the optimal dosage of this hormone.

Effect of Additional Estrogen Following an Initial Injection of Estrogen Adequate to Condition Animals for an Estrogen-Progesterone Induced Heat

Seventeen animals were injected with 10 R.U. of estradiol benzoate. Five came into heat on the estrogen alone. Ninety-six hours after the initial injection 6 of the 12 animals which did not come into heat received 20 R.U. of estrogen. At the same time the 6 remaining animals were injected with 0.4 I.U. of progesterone. The 6 which received the progesterone came into heat 3 hours after injection, while none of the 6 receiving the additional estrogen had come into heat within 9 hours, when observations were stopped.

When the data presented above are considered as a whole, the importance of progesterone for the induction of heat will be apparent. To be sure, the injection of estrogen is frequently followed by heat or by the other signs of a low grade 'estrous behavior,' but estrogen and progesterone acting synergistically were nearly always effective, even when small quantities were used. In the experiments described above, 137 animals were injected with from 5 to 100 R.U. of estrogen and observed well beyond the interval during which the

estrogen-induced heat is likely to occur. Of these, only 33 came into heat. Fifty-three of the remaining 104 were subsequently injected with 0.4 I.U. of progesterone. Of these, 51 displayed a normal heat response, and since the 2 in which heat did not occur were among those injected with the smallest quantity of estrogen, it is probable they were not properly conditioned. In the 31 animals in which 10 R.U. or more of estrogen was injected and did not cause heat, 0.4 I.U. of progesterone was effective in all.

The numerous observations of those who have studied morphological and behavior changes associated with reproduction, indicating that some animals are more sensitive to estrogens than others, are confirmed. Very early in the study animals were noticed which were more responsive to estrogen than others.

DISCUSSION

When the history of attempts to reproduce sexual receptivity in spayed rats is reviewed, the hypothesis that heat in this species is caused by the synergistic action of estrogen and progesterone is not irreconcilable with earlier observations. Allen, et al. (6), Hemmingsen (4), Ball (5), and Hemmingsen and Krarup (7) induced heat in spayed rats by injecting estrogens. The results, however, were never uniform because very large quantities were not effective in some animals while comparatively small quantities were effective in others. Other important observations were those of Witschi and Pfeiffer (2) and Ball (3) to the effect that mating in 'constant estrous rats' can be induced by the injections of luteinizing hormone and is associated with ovulation and corpus luteum formation. Cole (8) found that mating will occur in immature rats at the earliest ages at which ovulation can be induced regularly. The supposition which followed was that a factor supplementary to estrogen is involved, and as in the guinea pig (1), the action of progesterone seemed possible. The best evidence for the likelihood of this supposition has been the success with which heat was induced when progesterone was administered following a conditioning injection of estrogen. In a recent personal communication Doctor Ball has told us that she too has been able to induce heat in spayed rats by estrogen-progesterone treatment when the quantity of injected progesterone is larger than she used previously (3).

The possibility that heat in the rat is stimulated by the synergistic action of estrogen and progesterone suggests that the corpus luteum must be actively producing hormone at the time of its formation or even earlier in the non-pregnant animal. Morphological evidence for such activity is reported by Long and Evans (9). Physiological evidence, however, was lacking prior to the present work and Astwood's observation (10) that increase in water content of the uterus which is attributed to estrogenic action is inhibited prior to ovulation by the action of "an ovarian secretion having the properties of a corpus luteum hormone."

The data which have been presented suggest that the hormonal factors responsible for the occurrence of heat in the rat and guinea pig are essentially similar. However, certain quantitative differences exist. On the whole,

estrogen is more effective in causing heat in the rat than in the guinea pig. Injections of 1000 I.U. (200 R.U.) of estradiol benzoate induced heat in only 60% of the injected guinea pigs (1), whereas in the rat 100 R.U. of the same hormone caused heat in 90% of the animals. On the other hand, the rat appears to be less sensitive to progesterone. One-tenth of an international unit is an effective quantity of progesterone in the guinea pig, whereas the rats we employed did not respond regularly until 0.3 I.U. to 0.4 I.U. were injected. This species difference in response to corpus luteum hormone is also indicated by the results from studies of the effect of progesterone on ovulation and heat in normal animals. Dempsey (11) found that 0.05 I.U. of progesterone daily would inhibit ovulation and heat in the guinea pig, while Phillips (12) found that 1.5 I.U. per day was necessary to inhibit ovulation and heat in the rat.

SUMMARY

A small injection of estrogen followed after a suitable interval by an injection of progesterone is more effective than a larger quantity of estrogen alone in inducing sexual receptivity in the spayed female rat. With respect to length and intensity, the estrogen-progesterone induced heat resembles that of normal animals more nearly than the estrogen induced heat. It seems likely, therefore, that in the normal rat as in the guinea pig sexual receptivity is caused by the synergistic action of estrogen and progesterone produced in the still unruptured follicles. The rat appears to be more sensitive than the guinea pig to estrogen-action and less sensitive to progesterone-action.

We wish to express our appreciation of the very helpful advice given by Dr. William C. Young during the progress of this investigation.

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INDUCTION OF MATING AND OVULATION IN THE CAT WITH PREGNANCY URINE AND SERUM EXTRACTS[1]

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LITTLE IS KNOWN about the normal process of ovulation in the cat. Whether it occurs spontaneously, requires the stimulus of coitus or comes about in both ways has not been definitely established. Gruelich (1) found that 9 out of 12 cats, which were in estrum and in which the vagina was stimulated mechanically, ovulated, but he lacked controls showing failure to ovulate spontaneously. Van der Stricht (2) believes, from many observations, that coitus is essential. Longley (3) reported that 5 unmated cats in estrum failed to ovulate. But 4 of a group of 10 which were mated likewise failed to ovulate although they may have been killed too soon. Manwell and Wickens (4) observed no ovulation in unmated cats killed during estrum.

The evidence for spontaneous ovulation is inconclusive. Bonnet (5) found a tubal ovum in a cat which had been segregated from the male. Hill and Tribe (6) recovered unfertilized ova from two animals. Evans and Swezy (7) reported that an incomplete study by Miss Sadler demonstrated that ovulation may occur without coitus.

That ovulation can be induced experimentally in the cat has been proved but pregnancy urine extracts have not been used with much success. Snyder and Wislocki (8) brought about ovulation in one pregnant animal but 9 other cats failed to respond to doses which were similar and which would have been adequate for the rabbit. Bourg (9) obtained luteinization following rupture of a few follicles with pregnancy urine extracts. Foster and Hisaw (10) reported that large intravenous injections of pregnancy urine, luteinizing pituitary extracts and 33% acetone-soluble crude pituitary extracts failed to bring about perceptible changes in the ovaries and uteri of 10 cats. But the use of purified water-soluble follicular stimulating substances from the sheep pituitary (FSH) followed on the fifth or sixth day by the same or by a luteinizing acetone-soluble fraction (LH) of the pituitary or by a combination of FSH and LH did effect follicular development and ovulation.

A colony of cats has been maintained in our laboratory for the purpose of obtaining fetuses of known age for the past 11 years. Observation of several hundred animals has shown that estrum rarely occurs in captivity during the

[1] The pregnancy urine and pregnancy serum extracts were furnished by Parke, Davis & Co. and The Upjohn Company. I am especially indebted to Dr. George F. Cartland of the latter company for generous cooperation and for advice regarding the use of gonadogen in the present experiments, and to Mrs. Marian Sweet, Miss Dorothy Nelson, Mr. R. F. Becker and Mr. A. G. Steele for their assistance at various times during the course of these experiments.

season beginning with July and ending with January. Most spontaneous matings occur in March, April and May. We shall consider February to June, inclusive, the estrous season. In the hope that we could increase the number of timed matings during the estrous season and obtain fetal material in other months in which all animals are normally anestrous, we began injecting pituitary-like substances two years ago. Initial success with pregnancy urine and serum extracts lent encouragement although many failures were subsequently encountered.

METHODS

This report deals with results obtained in 122 cats, 48 of which were given the human pregnancy urine extract antuitrin-S (PU)[2] and the remainder, the mare pregnancy serum extract gonadogen (PS).[3] The animals were quartered in individual cages. Their diet consisted of whole milk and a mixture of ground beef hearts and Purina dog chow together with cod-liver oil. Not all animals which failed to become pregnant as a result of the treatment with gonad stimulators were killed. Many were given two and even three or more series of injections with several weeks elapsing between them. Principal attention will be paid the results of the first treatment. The ovaries and parts of the uteri of cats killed after various dosages of the extracts had been injected were preserved and some of this material was prepared histologically.

For the most part, the extracts were administered subcutaneously although in a few instances the intravenous or intramuscular route was used for the PS. There appeared to be no significant difference in results which could be attributed to the method of administration.

Vaginal smears were used to determine stages of the estrous cycle. Cats which showed no inclination to mate and whose vaginal smears contained scanty leucocytes and squamous cells were considered to be in the anestrum. All animals were observed for 10 days or more before treatments began and none came into heat spontaneously during this period.

OBSERVATIONS

Results with PU. One anestrous and 2 pregnant cats were given single subcutaneous doses of 100 R.U. The anestrous cat developed no vaginal change and no growth of ovaries or endometrium occurred. One pregnant animal was killed on the fourth day; the ovaries contained many large ripe follicles. The other, killed on the seventh day, had ovulated very recently.

Five anestrous cats received single doses of 100 R.U. followed in 4 or 5 days by 50 R.U. (3 cats) or by 3 U.U.[4] of PS (2 cats). The first 3 were killed on the tenth day. Two had developed marked squamous vaginal smear pictures and had ovulated; the third failed to show any vaginal change and the ovaries and uterus appeared to be entirely anestrous. The 2 cats receiving a supplementary dose of PS developed the characteristic estrous change in the vaginal mucosa and mated. They were allowed to live; but 20 days after mating, at which time it is easy to palpate the gravid uterus, no pregnancy could be determined.

Forty anestrous cats were given doses of 50 to 100 R.U. daily or at intervals of 2 days (some longer intervals in a few). The total dose varied be-

[2] Parke, Davis & Co.
[3] The Upjohn Company. In most experiments, lot Res. 44GFC18 was used.
[4] Upjohn units.

tween 150 and 450 R.U. Eight exhibited no change in the vaginal mucosa during the ensuing 2 weeks. One cat developed a squamous vaginal smear and typical estrous behavior but was not allowed to mate and when killed on the eighth day marked recent ovulation was found. The remaining 31 animals developed estrum and mated 4 to 8 days after beginning the injections (usually 5 or 6 days). Four were killed and were found to have ovulated (three unsegmented tubal ova were recovered in one instance) or to have developed heavily luteinized follicles. Sixteen became pregnant but 3 resorbed their fetuses. In the other 11 cats, no pregnancies could be palpated and no observations were made upon the ovaries.

When consideration was given to the time of year at which PU was injected it was observed that a larger percentage of successful experiments were obtained in the season during which cats normally came into heat in captivity than at other times. From the first of February to the end of June, 17 anestrous cats were treated. All showed estrous changes in the vaginal mucosa, most showed some swelling of the vulva, all exhibited willingness to mate and in 12 of them (70%) marked follicular stimulation, ovulation or pregnancy was proved. Twenty-nine anestrous cats were treated in the period we are designating the anestrous season from the first of July to the end of January. Ten showed no significant change in the vaginal mucosa and refused to mate. Nine developed some of the characteristics of estrum but failed to become pregnant and the ovaries were not examined. In only 10 cats (34.5%, the two pregnant animals excluded), did we encounter development of large follicles, ovulation or pregnancy.

Results with PS. Twenty-six cats received single doses of gonadogen subcutaneously (13 animals), intramuscularly (4 animals) or intravenously (9 animals). Three to 6 U.U. were employed in 17 of the cats and 9 to 18 U.U. in the remainder. The vaginal smears of 2 cats taken before injection of 6 U.U. were full of round, nucleated, epithelial cells indicating that these animals were already close to estrum; they mated on the second day but failed to become pregnant. Two cats given 6 and 10 U.U. were pregnant at the time; both came strongly into heat. They were killed on the eighth and seventh days after injection and the ovaries were full of ripe unruptured follicles. Twenty-two anestrous cats remained in this series. Six failed to show any vaginal change after 3 to 6 U.U. had been injected; two of the 6 were killed 8 to 10 days later and normal anestrous ovaries and uteri were found. Sixteen cats developed characteristic squamous vaginal smears but in some of them heat was not very strong. All mated. Six became pregnant (4 to 6 U.U.), 3 failed to become pregnant (6 to 18 U.U.), and 7 were killed at various times after mating (3 to 15 U.U.). Only one of the latter 7 cats showed any significant follicular growth and in this it was slight. This one animal received 6 U.U. of PS.

Twelve anestrous cats received one dose of 5, 6 or 8 U.U. followed in 5 or 6 days by 1.5 to 6 U.U. (total: 6.5 to 12 U.U.). Six of these animals (9 to 11 U.U.) were watched for willingness to mate but were prevented from doing so; vaginal smears were avoided. All came into heat 6 to 9 days after the first injection, remaining in heat until the twelfth day. They were

killed on the fourteenth day. All showed some follicular growth which, however, was slight in 2 animals. Marked follicular development and a single rupture point were observed in one, 2 ovulations in another cat. Complete ovulation of all ripe follicles (14 rupture points) was encountered in only one of the 6 animals. The other 6 cats of this series developed squamous vaginal changes and mated even though heat was not always strong but only 2 became pregnant.

Thirty-six cats received repeated subcutaneous doses daily or on alternate days. A total of 12 to 45 U.U. (usually 15 to 25 U.U.) was given. One pregnant animal (21 U.U.) developed strong estrous responses and, when killed on the twelfth day, was found to have ovulated. Four anestrous cats showed no vaginal change and one gave a very slight response. None of these 5 (33 U.U. in 10 days) would mate. Laparotomies were performed on the fourteenth day and the only animal in which any ovarian development appeared was that showing the slight vaginal change. Thirty anestrous cats developed squamous vaginal smears (usually on the 5th, 6th or 7th days of treatment). Not all exhibited swelling of the vulva. Three of them showed very slight heat and failed to mate as did 3 exhibiting strong responses. Of these 6 failing to mate, 4 were killed, one at 6 days and 3 at 8 days or more. That killed at 6 days exhibited marked follicular development, one killed at 8 days was ovulating at the time and the other 2 either had ovulated or had developed large follicles which had become luteinized. Twenty-four cats were mated. Two of these died and were disposed of in the absence of the author. Ten became pregnant. Twelve cats failed to become pregnant; 5 of them were killed and marked follicular stimulation, ovulation or luteinized follicles were found.

Seventy-four cats were treated with PS. Three were pregnant at the time and in 2 others the vaginal smears did not show the picture typical of anestrum. Of the 69 cats showing anestrous vaginal smears, 37 were treated in the anestrous season and 32 in the estrous season from February to July. Among the former, 10 exhibited no vaginal changes and failed to mate, 6 mated but examination of the ovaries disclosed no follicular growth, 5 mated but did not become pregnant, 3 showed only slight follicular development, and in 13 (35%) large follicles, corpora lutea or pregnancies resulted. Among the 32 anestrous cats treated in the mating season, all developed squamous vaginal changes, 11 (including 2 which died and were disposed of in the author's absence) mated but did not become pregnant, one showed slight ovarian growth and 20 (63%) exhibited marked follicular enlargement, ovulation or became pregnant.

Results with repeated treatments. Some cats which failed to respond to the original treatment with PU and PS were reinjected after rest intervals varying from several weeks to several months. Thirty reinjection experiments were performed with PU. One pregnancy resulted. Only 5 cats were killed; in 3, ovulation or luteinization of follicles was found to have taken place. Fifty-four reinjections were made with PS. Five pregnancies resulted. Thirteen cats were killed at various times after treatments. Of these, 5 had ovulated (few rupture points), one showed marked follicular growth, but

the remaining 7 had undergone no development of ovaries or endometrium.

One cat was given 10 series of injections in a little over a year and ultimately came into heat spontaneously after it had repeatedly failed to respond to the injected material. The accompanying table 1 summarizes the data for this animal. It will be seen that the cat was refractory to PU and PS when injections were spaced 5 weeks or less apart. Twelve other cats received 3 to 7 series of treatments at intervals of 2 to 5 weeks. In all but 2, there was similar evidence that some sort of refractoriness had been acquired to the material injected. Other cats receiving only 2 series of injections gave negative responses 2 to 5 weeks after one previous treatment which had been effective in producing estrous changes but not pregnancy. At autopsy it was found that the ovaries and uteri had undergone no growth as a result of more recent injections. Most administrations were made subcutaneously.

TABLE 1. CONDENSED RECORD OF RESULTS IN CAT 97

Date	Extract and dosage	Results		
		Estrum	Mating	Pregnancy
2/22/37	20 PS	+	+	−
4/13/37	45 PS	+	+	+[1]
7/12/37	15 PS	+	+	−
8/31/37	Spontaneous	+	+	−
10/ 8/37	110 PU	+	+	−
11/16/37	18 PS	−		
12/11/37	250 PU	−		
1/ 6/38	100 PU 3 PS	?	−	
1/26/38	30 PS	−		
2/ 7/38	12 PS	−		
3/ 7/38	30 PS	−		
4/14/38	Spontaneous	+	+	+[2]

[1] Uterus packed with fetuses on 5/26/37. By 6/10/37 they had been resorbed or aborted and eaten.
[2] Three fetuses at operation 6/1/38.
? Slight change in vaginal smear; no heat.

Hyperovulation and hyperfetation. We were unable to establish a minimal dosage of PU or PS which would consistently give uniform results. An amount which was adequate for ovulation in one cat was apparently inadequate for another. An amount which led to extensive follicular enlargement and luteinization without ovulation in one would bring about ovulation promptly in another. In a general way, however, it was observed that the larger the dose the greater the number of ovulations and pregnancies in each susceptible cat. Similar variability of individuals to standard doses of PS was observed by Hartman (11) in monkeys. At autopsy we commonly encountered ovaries with 8 or more corpora lutea in each.

Excessive numbers of fetuses were often found in the uterus. The maximum number was 13, whereas 4+ is the average in animals mating spontaneously. On a number of occasions, large numbers of vesicles (10 or more) could be palpated in the uterus on the 20th day after mating. None of the animals was allowed to go to full term, the fetuses being used for other experiments at various times in the gestation period. A high incidence of resorption, abortion and dead fetuses was encountered.

DISCUSSION

The present study demonstrates that anterior-pituitary-like substances of both pregnancy urine and pregnancy serum when given in suitable doses can be effective in initiating the reactions necessary for follicular growth, ovulation and mating in the cat. Although we failed to observe the ovaries directly before treating the animals, interpreting the estrous cycle solely by means of vaginal smears, we believe that the incidence of extensive follicular growth, ovulation or pregnancies within a limited time after treatment is too high to be fortuitous. All cats were observed for at least 10 days before the gonad stimulating substances were injected and none of them came into heat spontaneously.[5] Of the 115 cats showing anestrous vaginal smears, all but 20 developed an estrous picture after injections of PU or PS; 36 mated but showed no follicular growth or were not killed, 4 exhibited slight follicular development and 55 (nearly 48%) were found to have developed many large follicles, ovulated or become pregnant (34 became pregnant) at the time expected experimentally. All 5 cats pregnant when receiving PU or PS showed follicular growth or ovulation afterward.

Had we been dealing with small numbers of cats our results might have been very different. We began with a group of 10 cats, treating them in February; 8 became pregnant. However we had other series of 10 or more animals at other times of the year from which no pregnancies could be obtained. Altogether there were 66 anestrous cats treated during the anestrous season and 49 in the estrous season. Forty-six of the former developed the vaginal estrous picture and all the latter did so but 2 of them would not mate. Three cats in the anestrous, and one in the estrous season showed slight follicular enlargement. Twenty-three developed large follicles, ovulated or became pregnant in the anestrous, and 32 in the estrous months. Twenty-four of the 34 pregnancies were obtained in the estrous season and 7 others were in the latter part of January or the early part of July, i.e., at the end or beginning of the anestrous season. These data signify that pregnancy urine and serum extracts were more effective when used at the time of year one might expect animals to mate normally than they were at other times. Herein seems to lie possible reason for other investigators' failures to bring about ovulation in cats with the gonad stimulators. The season factor had not been suspected. No explanation of this seasonal refractoriness is forthcoming from the present experiments. However, we have some more recent experiments in which anestrous cats in the anestrous season failing to respond to gonadogen did develop estrum when treated with the Fevold-Hisaw FSH of the sheep pituitary gland. These and other incomplete observations with FSH seem to show that the seasonal refractoriness applies to PU and PS but not to FSH (12).

One phenomenon encountered in the present series of experiments was the development of some sort of a refractory state by the administering of the PU and PS with the result that subsequent injections very often failed

[5] The two showing proestrous vaginal smears undoubtedly would have come into heat normally in a few days had they not been given treatment.

to elicit responses even in the season of the year when estrum was to have been expected. A similar refractoriness was reported in the monkey by Meyer and Gustus (13). One thinks of the possibility of antibody formation (14, 15), the hormones which in themselves may be proteins or attached to proteins acting as antigens. That this particular reaction may not have been concerned with an anti-hormone (16) is suggested by the fact that estrum, with successful mating and pregnancy, sometimes appeared spontaneously during the time the animals were refractory.

SUMMARY

Pregnancy urine or serum extracts (antuitrin-S and gonadogen) were employed in an attempt to induce estrum, mating and pregnancy in cats. Experiments were performed in 122 animals of which 5 were pregnant at the time and 115 were not pregnant but were judged to be anestrous from vaginal smears. Suitable doses of the two extracts were similarly effectual for follicular growth, ovulation and mating in anestrous and pregnant cats which had not been treated previously with these materials. Induction of estrous vaginal smears, estrous behavior, and the attendant follicular growth, ovulation and pregnancy were more readily accomplished with these substances during the time of year at which cats in captivity enter estrum spontaneously (February to June, inclusive) than during other months. Squamous vaginal smears, with manifestations of heat and with matings, sometimes appeared as results of treatments but without any grossly visible ovarian and endometrial development especially during the anestrous season. Great variation in the effectual dose and no difference between intravenous and subcutaneous treatment were encountered. Hyperovulation and hyperfetation resulted in the instances in which suitable quantities of the materials were injected to bring about satisfactory matings. Retreatment within 2 to 5 weeks after initial doses had been given was frequently ineffectual, the animals apparently becoming refractory in consequence of the earlier injections. Such refractory animals sometimes came into heat spontaneously, mated and became pregnant.

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TECHNIC FOR HYPOPHYSECTOMY OF PIGEONS[1]

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THE EXTENSIVE use of hypophysectomized pigeons in the study of anterior pituitary functions and in testing hormone preparations has made it desirable to develop for these animals a reliable and rapid operative technic. The procedures of earlier workers (1, 2, 3) were tried and certain features of their methods adopted. The procedures and instruments which serve best in our hands after much experience are here described in detail.

The earlier experience of Dr. C. S. Moran in this laboratory (unpublished) indicated that both the orbital approach (4, 5) and the transbuccal approach (1, 2) involve danger of infection and interference with the feeding mechanisms. Although pigeons are notably resistant to bacterial infection many birds which lived for some weeks after operation developed protozoan (*Trichomonas sp.*) infection at the operative site, and these infections were often the apparent cause of death. The parapharyngeal approach (3), suitably modified for the pigeon, was adopted and has proved to be a most satisfactory procedure.

EQUIPMENT

The bird is fastened to the operating board by elastic strips cut from automobile inner tubes. A longitudinal strip on each side holds right and left wings; a transverse strip, divided in the middle, holds the feet. A head-holder fashioned from a block of cork to fit the contours of the bird's head is fastened near one end of the board (fig. 2). It is essential that the head be held in such position that the floor of the skull is horizontal. The beak fits into a vertical groove in the head-rest and is held there by being inserted between the cork and an elastic band wrapped around the top of the holder. To prevent the bird from withdrawing its head posteriorly, a wicket-like support is attached to the board so that it impinges against the occiput. Upward movement of the head is prevented incidentally by application of tissue retractors after dissection has been started. These retractors are hooks made of wire attached to the head end of the board with elastic bands at points determined by the amount of tension needed.

Dental plug pliers[2] are used for dissection because they have suitably

[1] Aided by a grant from The Carnegie Corporation of New York.

[2] BONE WAX—Horsley's aseptic. Kny-Scheerer Corp., Long Island City, N. Y. (No. 2777). COTTON PELLETS—Assorted sizes 0 to 3. Richmond Dental Mfg. Co., Niagara Falls, N. Y. DENTAL BURRS—assorted sizes. Cost about $1.00 per doz. DENTAL ENGINE—Type 10 portable. Foredom Electric Co., New York, N. Y. DISSECTING FORCEPS—Perry annealing pliers. S. S. White Dental Mfg. Co., New York, N. Y. DISSECTING NEEDLE—Dental explorer, single-end, long handle. S. S. White (No. 2). Cost $1.00. DISSECTING SPUD—Risley's eye spud, angular. Kny-Scheerer (No. 3149). NEMBUTAL—Abbott Laboratories, North Chicago, Ill. RETRACTOR—Buckley tissue retractor, H. Fischer and Co., New York, N. Y. (No. 281).

curved fine tips and are long enough to be held in the hands like pencils. Drilling is accomplished by means of a portable electric dental drill with cable drive controlled by a foot treadle. The most useful sizes of burrs for making the drill-hole in pigeons are numbers 7 to 10 (2 to 2½ mm.) depending upon the size of the bird used.

Some kind of aspirating device (suction pump) is needed to remove fluid and tissue from the drill-hole. A suction flask is placed in the vacuum line to keep the fluid and tissue out of the pump and to permit recovery of the gland

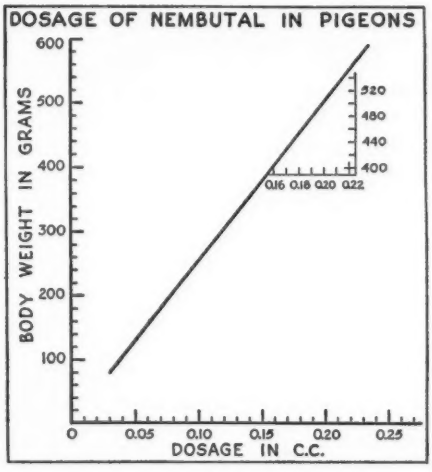


Fig. 1. THIS DOSAGE CURVE APPLIES TO INTRAVENOUS INJECTION OF NEMBUTAL (ABBOTT) SOLUTION, 1 GRAIN PER CC. The lower part of the curve applies to squabs and ring doves; the upper part to sub-adult and adult pigeons. Although this average curve is a straight line and is generally applicable, certain modifying conditions may be encountered; for example, an adult bird of 150 gm. body weight may require as much as 50% more of the drug than a squab of the same weight.

at the end of the operation. The suction cannulae are easily made from stock glass tubing by drawing out the tip to a diameter suitable to the size of the gland to be passed through it. A small hole is made in the side of the cannula where it can be closed by the finger when suction at the tip of the cannula is desired; the chief advantage of the side-port lies in the fact that when the port is open, the cannula can be used as a probe or retractor during certain stages of the operation. It is especially desirable to use the end of the cannula for tamping the bone-wax into the hole left by removal of the gland.

OPERATIVE PROCEDURE

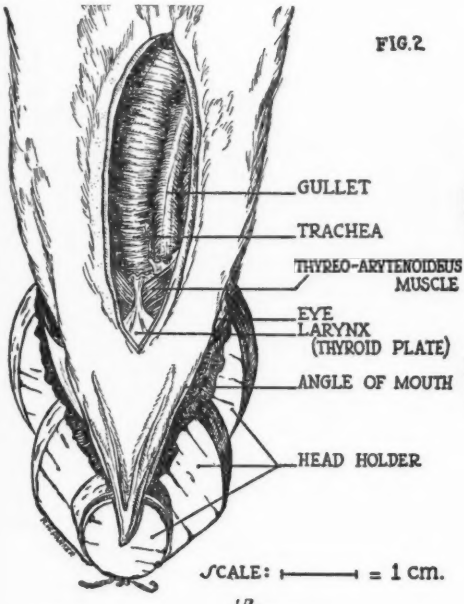
Anesthesia. Parenteral anesthetics are superior to pulmonary types for use in the bird. The bird is weighed and injected intravenously with nembutal at a level of 25 mg. per kg. of body weight (see fig. 1). Injection is usually made into the radial vein which is easily exposed by plucking a few feathers under the wing and is large enough to admit a 26-gauge needle

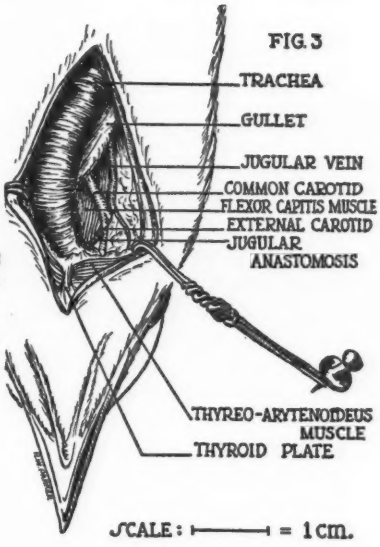
without difficulty. If the injection is prolonged over about one-half minute, the anesthetic is tolerated with little shock. By the time the injection is complete anesthesia is deep enough to permit immediate operative procedure.

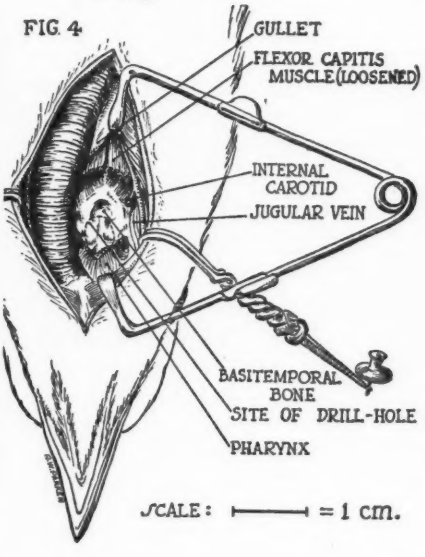
The feathers are plucked from a strip about 1.5 × 4 cm. under the neck, beginning at the base of the lower beak. The bird is then placed (back downward) on the operating board, and wings and feet fastened by means of elastic bands. The head is placed in the head holder by slipping the upper beak under the encircling rubber band and the neck is made straight by pushing the body postrad.

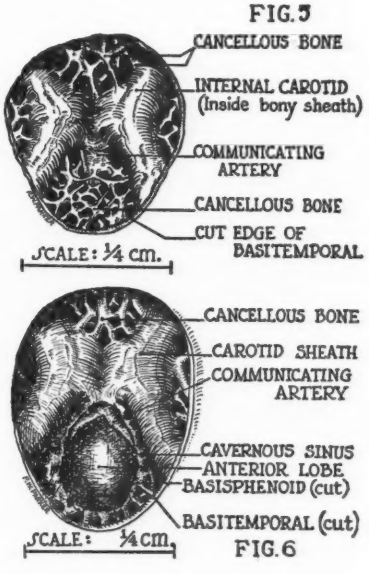
Dissection. Beginning above the angle of the mouth, a longitudinal incision about 3½ cm. long is made through the skin and platysma of the neck. The fascia and sternohyoideus muscles overlying the trachea are separated with forceps until the trachea is bare throughout the length of the incision. The trachea is displaced slightly to the left side and the gullet exposed. By blunt dissection the gullet is separated from its adjacent tissues on the right side (fig. 2). Dissection is continued until the jugular anastomosis appears as a large transverse vessel lying above the flexor capitis inferior muscles. The right forceps are now passed under the trachea (between trachea and gullet) just posterior to the larynx and allowed to spread. This makes an opening through which the left hook retractor is passed and hooked over the gullet. The right hook retractor is then adjusted over the skin, lateral muscle bundles and connective tissues deflecting them toward the right (fig. 3). It is now possible to see the transverse connecting jugular vein and the two bands of the flexor capitis inferior muscles enveloping the floor of the skull. By grasping a cotton pellet in the forceps and sliding it with gentle pressure along the skull the muscles and periosteum are stripped from the bone. This step usually breaks a small vein emerging from the middle of the skull and passing between the longitudinal muscle bands to the superficial tissues. If troublesome bleeding results the free end may be caught under the retractor (see next step). Bleeding from the emergent end is stopped when drilling has proceeded far enough to release the vein from the bone surrounding it.

A spring tissue retractor is now put in place in the anteroposterior plane, the forward prongs placed against the transverse jugular connection, and the posterior prongs hooked under some of the loosened muscle and the fascia above it. Care must be taken that the forward prongs do not compress the larynx enough to throttle the bird and yet retract sufficiently to hold the trachea slightly to the left of the midline. With the retractors all in place it remains to clear the skull completely of tissue so that the landmarks are visible and there is nothing to interfere with the drill. A median eminence of the basitemporal bone just posterior to its junction with the rostrum is the most important point to locate. The triangular surface of the basitemporal should be cleaned beginning with the anterior prominence and extending postrad 7 to 8 mm. (fig. 4). From this point onward the work is aided by magnification. We use a Greenough dissecting microscope with magnification of 10 diameters. This enables us to avoid blood vessels which might otherwise be ruptured and also to control our instruments with much greater









Figs. 2–6

precision. Although the operation can be done with little or no magnification, we find that its use means the difference between chance and certainty in the result. The operative field is illuminated by means of a focusing spot-

light of The Spencer "Universal" type mounted on the microscope in such a way that the light beam is nearly parallel with the optical axis.

Drilling. Drilling is now begun by placing the burr on the eminence and moving it with a slight circular motion as the drill rotates. The solid plate of bone is soon penetrated and the burr sinks into the cancellous bone beneath. The circular movement of the drill should be continued, enlarging the opening in the solid bone to an elliptical hole of about 4 ×6 mm. (the full width of the basitemporal) making sure that the hole extends to the anterior tip of the basitemporal. The drilling may now continue on the cancellous bone using care not to strike the paired internal carotid arteries which pass obliquely beneath the lateral margins of the drill-hole (fig. 5). Progressive removal of the cancellous bone which surrounds these vessels allows them to separate sufficiently to give working room between. At the fork of the carotids it will be seen that a dorsal twig is given off which extends vertically through the cancellous bone away from the observer. By following this twig with the drill (to a point about 4 mm. below the surface of the basitemporal) it is seen to enter a bulge in a second layer of hard bone. This bone is the basisphenoid which is ankylosed with the more superficial basitemporal to form the floor of the skull.

The bulge penetrated by the carotid twig is the sella turcica. From all around this eminence the cancellous bone is cleared away; and, with a circular motion of the drill (following around the periphery of the sella) a thin disc of bone is loosened, and then removed. The dural sheath of the pituitary should now be visible bulging up through the hole in the basiphenoid (fig. 6). The bone surrounding the gland should be explored to make certain that the whole anterior lobe is free. The anterior lobe does not lie in the horizontal plane but slopes downward anteriorly. Special effort should be made to expose the deeper-lying anterior end. An eye-spud is a convenient instrument for use in this exploration and for trimming the bone where necessary. A small amount of bone-wax is now rolled between the fingers to make a tapering plug about 3 × 10 mm. (these are best made in advance). If this plug is placed on the tissue back of the drill-hole it will become soft from the heat of the bird's body and be in position convenient for use when needed.

Removal of the gland. The suction cannula is now taken in the left hand and the dura hook in the right. Beginning at the anterior margin of the exposed dura a slit is started and continued backward to the fork of the internal carotid twig (posterior communicating arteries) which is seen at the posterior end of the anterior lobe (fig. 6). If this operation is successful the anterior lobe will bulge up through the slit and very little bleeding will result. The forked internal carotid passes around the posterior end of the anterior lobe which it often embraces tightly enough to produce a stricture. It is necessary to displace the carotid slightly upward and backward in order to free the anterior lobe completely. This is usually done as the dural slit is completed. Profuse bleeding may result from opening the cavernous sinuses, particularly while the carotids are being manipulated. Application of suction to the gland will elevate it against the sides of the opening and stop the hemorrhage. At this point the gland is sufficiently exposed to permit

removal by suction. The white glandular tissue can be seen filling the end of the cannula as sufficient negative pressure is built up to pull it free. With a sudden rush it comes loose and passes up through the cannula into the suction flask, followed by a gush of blood. As suction is continued momentarily, the empty sella can be seen with the white posterior lobe showing through at the posterior-dorsal end of the sella beneath the fork of the communicating carotids.

Closing the wound. The anterior lobe usually comes out in one piece; thereafter it is merely necessary to stop the hemorrhage and close the wound. The hemorrhage can be controlled by cotton compresses but better by the plug of bone-wax (tamped in lightly with cannula and forceps). No infections, harmful localized pressures, or other evidences of injury result from the use of this wax. The wax is left in place, the spring retractor is removed, the lateral hooks are taken out, and the skin incision sutured.

Clearing the trachea. The mouth should be brushed with dry cotton to remove the mucus which has accumulated there. While the bird is on its back the mucus falls away from the glottis and therefore causes no difficulty; but if normal posture is resumed while mucus is in the throat this is immediately drawn into the trachea with an occasional fatal result. The bird is released, keel down, in a warm place to recuperate for a few hours. No further attention need be given to the operated birds unless breathing is accompanied by a marked rattle in which case the trachea should be cleared with the suction cannula.

Recovering the gland. If bleeding has been profuse so that the fluid in the suction flask is quite red, it is better to clamp the tube leading to the cannula so that continued evacuation hemolyzes the blood. This breaks up the clots and makes the gland easier to find when the contents of the flask are poured into a flat dish. The gland should be examined to see that it is whole and unbroken.

Post-operative care. If left undisturbed after operation the birds remain quiet for 3 to 5 hours. They need no particular care other than to be protected from sudden changes in temperature; they seem to fare best at or above 70° F., and should have access to perches. Their spontaneous activity decreases after the operation and they commonly sit with ruffled feathers during most of the day; during the succeeding days their food and water consumption is decreased along with a marked loss in body weight.

The post-operative mortality is low, and birds may be expected to live for one or more years. Occasional birds die after two or three weeks apparently from some deficiency connected with hypophysectomy. Some birds exhibiting convulsions resembling hypoglycemic shock have been temporarily supported by glucose administration; but such cases, if detected early, can be better managed by an injection of whole anterior pituitary extract.

DISCUSSION

When this technic has been mastered, and with the help of an assistant to anesthetize and handle the birds, 5 to 8 operations can be done in an hour. The eustachian tubes can be avoided by the parapharyngeal approach but

they are usually destroyed in our routine operations. Most of our operations have involved the removal of the anterior lobe only; but both lobes can be removed by packing the sella with wax after removing the anterior lobe, pushing it aside and using a smaller cannula to aspirate the posterior lobe. The posterior lobe alone can likewise be removed by rotating the anterior lobe to the left and aspirating with a fine cannula. In any case there is little likelihood of injuring the brain for reasons cited by Hill and Parkes (1).

Barring accidents in the operative procedure the mortality during operation is very low. Our loss is less than 2% from this cause. Some birds (less than 1%) seem intolerant of nembutal and die immediately after injection of the anesthetic. In a series of examinations made after 10 days on 230 large birds of uniform stock and age (young) less than 5% showed pituitary remnants demonstrable by sectioning the sella (including posterior lobe, diaphragma and stalk). Most of these had less than the 5 to 6% of gland necessary to produce an effect on organ weights (unpublished data of this laboratory). Operated adults and nestlings allowed to live for longer periods have shown a somewhat higher percentage of incomplete removal. Whether this results from faulty technic incident to the smaller body size of some of these birds, from regeneration of unremoved fragments, or from other unsuspected cause is now unknown.

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INFLUENCE OF PROLONGED ETHERIZATION, TRAUMA AND HEMORRHAGE UPON THE SURVIVAL PERIOD OF ADRENALECTOMIZED RATS

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IN AN EARLIER communication (1) dealing with the influence of environmental temperature and salts upon the survival period of adrenalectomized rats, we mentioned a number of additional factors which may influence the survival period of such animals. In the present paper we have investigated the factors of etherization, trauma and hemorrhage.

In studies on adrenal function in which adrenalectomized animals are employed it is obvious that factors which complicate or influence post-operative behavior and survival should be thoroughly understood in order to ascertain the true function of the adrenals. The importance of such an understanding has been stressed by various investigators including Stewart (2) whose discussions deserve attention. In his excellent review he emphasizes the importance of careful operative procedures. It is his belief that " . . . the shortening of the operation including the anesthesia, and the diminution of the effects of trauma . . . " are factors which tend to prolong the survival period of adrenalectomized animals. He calls attention to the " . . . ease with which animals, especially small animals, can be killed by bad surgery, with its concomitants and consequences, excessive anesthesia, hemorrhage, trauma, cooling and the shock to which all these factors contribute." He states that "when animals die in a day or so after adrenalectomy the loss of adrenal function, if it contributes at all to the fatal result, is of far less importance than the general effects of the operation."

Schultz (3) in a brief statement involving unpublished work on rats stressed the importance of properly administering ether during the operation which he says "reduces the side actions of an ether adrenal operation to a minimum."

Firor and Grollman (4) in a study of adrenalectomy in mammals stated that trauma, hemorrhage and anesthesia are factors which shorten the lives of adrenalectomized animals. They stressed the importance of celerity of adrenal operations, especially with ether anesthesia. In adrenalectomy of rats they found that lengthening of the etherization from 5 to 10 minutes greatly shortened the post-operative survival period. In experiments on rats and dogs they reported that animals anesthetized during adrenalectomy with amytal and the spinal anesthetics novocain and tutocain lived longer than animals anesthetized with ether.

Freed (5) found that post-operative trauma produced in 30 to 40-day old rats by crushing the hind leg muscles at from 18 to 36 hours following adrenalectomy resulted in the death of the majority of such animals in from 1½ to 12 hours. He also found that death of these animals could usually be prevented by the subcutaneous injection of saline solution.

Swingle and Parkins (6) in an extensive study of trauma in adrenalectomized dogs found that limited post-operative trauma in healthy vigorous adrenalectomized dogs with well-healed operative wounds resulted in rapid and fatal shock whereas the effects in normal dogs were negligible. This susceptibility of the adrenalectomized dog to trauma was found to be corrected, at least in part, by the administration of large doses of cortical hormone. The authors pointed out that when trauma is combined with prolonged anesthesia in either normal or adrenalectomized dogs the shock symptoms are more severe and persisting than in animals which are allowed to gain consciousness quickly following trauma. They demonstrated that the recovery from shock in the conscious animal results from the ingestion of large quantities of water which restore the low volume of circulating fluid depleted by losses into the injured tissues. It was also made apparent by their work that such recovery from shock, even with copious intake of fluid, is impossible unless adequate cortical hormone is present.

With respect to the methods of producing trauma the authors found crushing of the testes inadequate for producing shock in unoperated animals even when hemorrhage was superimposed. They also found that the intestinal stripping necessary to produce shock in normal dogs was so damaging as to make their revival useless and with respect to muscle trauma to the hind limbs: "It is difficult to quantitate the degree of trauma by this method so that each animal receives a comparable amount of tissue abuse."

With respect to the common observation that one-stage bilateral adrenalectomy in dogs usually results in death within 24 hours, the authors state that their various experiments provide evidence which indicates that operative manipulations accompanying adrenalectomy are relatively unimportant in inducing shock regardless of the extent of the associated trauma. They believe that the shock resulting from bilateral extirpation at one sitting is due to the complete and sudden loss of all reserve cortical hormone.

Rich (7) in a study of etherization and traumatic shock in cats found that freshly adrenalectomized cats reacted to intestinal manipulation the same as do normals, whereas cats similarly treated 17 hours following adrenalectomy were shocked in ⅓ of the time. He found that lengthened (continuous) etherization before traumatization delays the onset of shock although once shock begins etherization aggravates it.

EXPERIMENTAL METHODS

The operative procedures employed and the general care of the animals were similar to those followed in previous work (1). All animals were maintained on a low salt intake at an environmental temperature of $22° \pm 2°$. Equal division of sexes and individual litters was made between experimental and control groups. The rats of litters used in any given experiment were all of nearly the same age.

Etherization. For most of the work Mallinckrodt's anesthetic ether was administered by the 'open method' by allowing the animal to breathe from the open mouth of a small vessel containing cotton soaked with ether. In several experiments in which etherization was maintained for long periods of time a closed method was used which proved efficient and safe. It consisted of an arrangement whereby humid air at 35° was mixed with controlled amounts of ether vapor and passed through a large anesthetizing chamber at a rapid rate.

Trauma. Various methods of producing trauma were tried but most of them were unsatisfactory because they were either insufficient or the extent of trauma could not be gauged with any reasonable accuracy.

The method which was finally adopted consisted of crushing the tissues of the anterior abdominal wall (immediately following adrenalectomy), with a small strong curved hemostatic forceps through the incisions made at the time of adrenalectomy. The base of the jaws of the forceps was cut away so that the edges of the wound were not traumatized. The lower jaw of the forceps was passed through the incision into the peritoneal cavity while the upper jaw remained on the exterior of the abdominal wall. The forceps were moved about to fresh tissue as the traumatization proceeded taking care not to injure the viscera. The forceps were closed to a definite point and for a given period of time at each application. This procedure proved to be very satisfactory for producing quantitative effects by varying the number of applications of the forceps. The blood supply was apparently not altered sufficiently to seriously limit nutrition to the tissues since in animals which lived the injured tissue rapidly healed.

Hemorrhage. Experimental hemorrhage was produced by placing the rats in a warm room for a short time and bleeding from the tail just prior to adrenalectomy.

RESULTS

A total of 478 young white rats was used in the experiments. A few of the bilaterally adrenalectomized animals which lived on for a period of one month or longer were killed and at autopsy revealed accessory cortical tissue. Data from these animals are not included in the tables.

In the first experiment a group of young rats were subjected to prolonged etherization following bilateral adrenalectomy. The results are shown in table 1. When compared with the group of control animals in which etherization was not continued beyond the period of operation it is evident that

TABLE 1. EFFECT OF PROLONGED ETHERIZATION UPON THE DURATION OF LIFE OF BILATERALLY ADRENALECTOMIZED RATS

No. of rats	Average weight	Period of etherization	Nature of trauma	Nature of operation	Duration of life in days			
					Range	Average	Median	Average deviation (median)
40	61.3	10 min.	none	Bilateral adrenalectomy	5–13	7.96	8.0	1.75
40	61.4	1–2 hr.	none	Bilateral adrenalectomy	4–14	7.9	7.5	2.53

the prolonged etherization produced no significant variation in the duration of the life of these animals. As a further test of the effects of prolonged etherization we have etherized rats for several hours at a 2-day post-operative period without apparent influence on survival.

The second experiment was concerned with the effect of severe trauma upon the duration of life of adrenalectomized rats. The trauma was produced by applying the forceps 20 times to different areas of the abdominal wall immediately following adrenalectomy. The results of this experiment are shown in table 2. The severe trauma to which these rats were subjected resulted in the death of the majority of the animals within a period of less than 20 hours which demonstrates that young rats cannot withstand severe traumatization at the time of operative removal of the adrenal glands.

TABLE 2. EFFECTS OF SEVERE TRAUMA UPON THE DURATION OF LIFE OF BILATERALLY ADRENALECTOMIZED RATS

No. of rats	Average weight	Period of etherization	Nature of trauma	Nature of operation	Duration of life in days			
					Range	Average	Median	Average deviation (median)
21	40.1	10 min.	severe	Bilateral adrenalectomy	(All died in less than 20 hours.)			
18	44.4	10 min.	none	Bilateral adrenalectomy	7–8	7.2	7.0	0.1
10	42.0	10 min.	severe	Unoperated	(All lived indefinitely)			

In order to determine the efficiency of traces of the glands in protecting against the lethal effects of severe trauma, an experiment was conducted in which a small portion of the adrenal glands (about 1/8) was left at the time of operation. These animals were severely traumatized along with controls at time of operation. The results as presented in table 3 show that small traces of the glands left at the time of operation are, in the majority of cases, fully protective against the effects of severe trauma fatal to completely adrenalectomized animals.

TABLE 3. EFFECTS OF SEVERE TRAUMA UPON THE DURATION OF LIFE OF PARTIALLY ADRENALECTOMIZED RATS

No. of rats	Average weight	Period of etherization	Nature of trauma	Nature of operation	Duration of life in days			
					Range	Average	Median	Average deviation (median)
28	43.0	10 min.	severe	7/8 adrenals removed	(all lived indefinitely excepting 3 which died in less than 20 hours.)			
29	41.0	10 min.	severe	Bilateral adrenalectomy	(all died in less than 20 hours.)			

TABLE 4. EFFECTS OF POST OPERATIVE TRAUMA UPON THE DURATION OF LIFE OF BILATERALLY ADRENALECTOMIZED RATS

No. of rats	Average weight	Period of etherization	Nature of trauma	Nature of operation	Duration of life in days			
					Range	Average	Median	Average deviation (median)
32[1]	40.1	10 min.	moderate	Bilateral adrenalectomy	5–8	6.3	6.5	1.8
30	41.2	10 min.	moderate 3 days after oper.	Bilateral adrenalectomy	(All dead less than 6 hours after traumatization on the third day.)			

[1] Control animals were etherized 3 days following adrenalectomy for the same period as the experimental group.

The next experiment, the results of which are presented in table 4, was planned to compare the effects of trauma produced at the time of operation

with post-operative trauma. It illustrates very clearly that adrenalectomized animals are much more susceptible to trauma 3 days following adrenalectomy that at the time of operation.

In the foregoing experiments the factors of trauma and etherization were studied singly and although these studies have clearly established the importance of the factor of trauma and the apparent unimportance of the length of etherization, they have not settled the possibility that prolonged etherization may assume importance when associated with other factors or combination of factors such as trauma, hemorrhage, etc.

Consequently we have conducted numerous and extensive experiments in an attempt to evaluate the influence of prolonged etherization when combined with trauma. In these experiments an endeavor was made to graduate the amount of trauma in such a manner that any effects of prolonged etherization would become apparent. Our results so far have been inconclusive. However, it appears that prolonged etherization even in combination with trauma, has little, if any influence on the survival period of adrenalectomized rats.

TABLE 5. EFFECTS OF HEMORRHAGE AND MODERATE TRAUMA UPON THE DURATION OF LIFE OF BILATERALLY ADRENALECTOMIZED RATS

No. of rats	Average weight	Period of etherization	Nature of trauma	Nature of operation	Duration of life in days			
					Range	Average	Median	Average deviation (median)
15	36.2	10 min.	Bled 1 cc. mod. trauma	Bilateral adrenalectomy	(All except 2 animals died in less than 24 hours.)			
15	35.0	10 min.	Bled 1 cc.	Bilateral adrenalectomy	2–7	5.0	5.5	1.6

We have also conducted experiments on the influence of hemorrhage on the duration of life of adrenalectomized rats. As yet investigations on the factor of hemorrhage alone have not yielded satisfactory data. However when combined with trauma the effects of hemorrhage become apparent as shown in table 5.

DISCUSSION

As reviewed in an earlier part of this paper, various investigators have considered prolonged ether anesthesia as being detrimental to survival of adrenalectomized animals. This may be generally true for most animals but from our experiments is not true of rats, especially young rats, which we have found to be able to withstand at least an hour of post-operative etherization without effect. There are other circumstances as yet uninvestigated which might explain the harmful effects of etherization which have been reported, such as upper respiratory infections, which, with prolonged etherization might result in anesthetic pneumonia or pre-operative anesthesia which at the hands of a clumsy operator may be prolonged before extirpation of the glands is completed.

Compared with other laboratory animals the rat is unique in its resistance to traumatic shock. This resistance to many and varied types of shock has been referred to by many investigators including Freed (5). The resistance of the normal rat to surgical shock seems to be transmitted, to some extent at least, to the freshly adrenalectomized rat which can withstand a considerable amount of trauma before being affected. This early resistance may be due to protective quantities of cortical hormone which may persist in body tissues for some time following adrenalectomy or to resistant mechanisms not dissipated for some time following adrenalectomy. At least, trauma 3 days after adrenalectomy is much more injurious than at the time of operation. The combined effects of hemorrhage and trauma proved more severe in producing shock than either alone. This was to be expected since both contribute to lowered blood pressure and circulatory collapse.

It is evident from our experiments that the trauma and hemorrhage accompanying bad surgery may contribute to shortening the survival period of adrenalectomized rats. However in the highly resistant rat, such abuse must be extreme in order to appreciably influence the survival period. From our observation we believe that, among experienced operators, such factors as freedom from infections, diet, and environmental temperature during both pre-operative and post-operative periods are in the majority of cases the most important influences determining the life span of adrenalectomized rats.

SUMMARY

Following adrenalectomy young white rats were subjected to the various conditions of prolonged etherization, trauma and hemorrhage. The results show that prolonged etherization following bilateral adrenalectomy has no influence on the duration of life of white rats whereas, severe trauma following bilateral adrenalectomy markedly reduces the duration of life of these animals. Adrenalectomized rats are much more susceptible to trauma produced three days after adrenalectomy than to trauma produced immediately following adrenalectomy. Traces of adrenal gland which remain following partial adrenalectomy serve to protect such rats against the lethal effects of severe trauma and in most cases permit their indefinite survival. Hemorrhage when combined with moderate trauma markedly reduces the duration of life of adrenalectomized rats.

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STUDIES ON THE RESPIRATION OF THE NEWT

II. THE EFFECT OF TEMPERATURE IN HYPOPHYSECTOMIZED, IMMATURE, THYROIDECTOMIZED AND PANCREATECTOMIZED MALES

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CAMBRIDGE AND WORCESTER, MASSACHUSETTS

In a previous study (1), a method employing a modified Warburg respirometer for the simultaneous measurement of the oxygen consumption and the carbon dioxide production of the newt, *Triturus viridescens* Raf., was described. Using this instrument it was found that the temperature characteristic (μ) for the respiration of normal animals was approximately 17,200. This value was not found to change as a result of gonadectomy, but μ values of 13,800 and 17,200 on either side of approximately 15° C. were found for fall-collected animals which had been kept under laboratory conditions without feeding for several weeks. It was concluded that this change was probably due to starvation.

The observations reported in the present paper deal principally with the effect of various experimental modifications of the endocrine complex on the temperature-respiration relationship as described by the temperature characteristic.

All experiments in this series were made on newts recently removed from the field and not fed during the course of the study. The technic used in the care of the animals as well as the method for the measurement of their respiration was essentially the same as that which was reported in the initial study (1).

Respiration Following Hypophysectomy

The pituitary gland was removed by the oral route. Its location can usually be made out readily as the tissue lying dorsal to a pinkish area in the posterior part of the roof of the mouth between the vomerine teeth. Under chloretone anesthesia the mucous membrane of the region was removed and the bone cut away with a sharp dental chisel. The gland was aspirated out of the cranial cavity by means of a glass pipette attached to a rubber tubing. Antiseptic precautions were not found to reduce the mortality to any noticeable degree. The animals were kept at room temperature. Blackening of the skin which accompanies the removal of the pituitary or the thyroid glands in newts (2, 3) served as a useful test of successful operative technic. All animals, however, were autopsied at the close of the experiments to insure that

regeneration had not taken place. Respiration measurements were begun about 6 weeks after operation when skin blackening and asthenia were well developed.

Arrhenius plots of the respiratory rate of 4 hypophysectomized individuals (fig. 1) at temperatures ranging from 8.6° to 27.7° C. showed no marked deviation from enclosing lines which were copied from corresponding plots

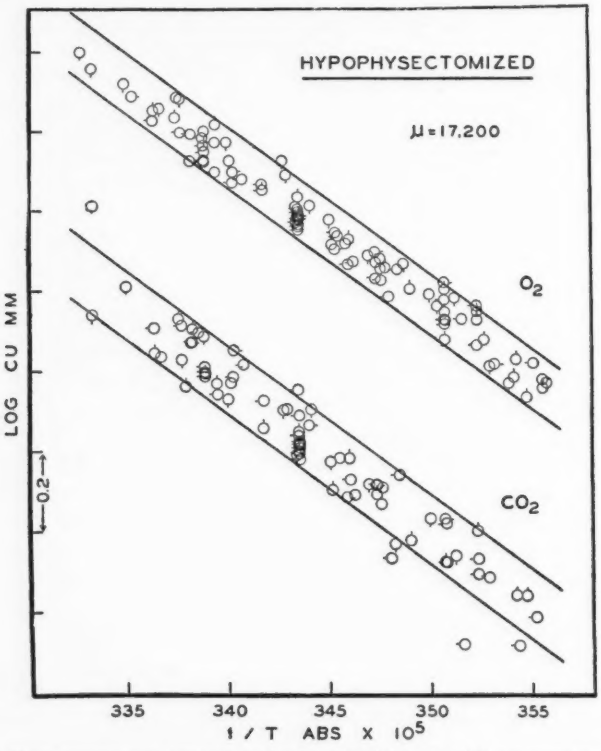

Fig. 1. Hypophysectomized animals. Arrhenius plot of the rate of O_2 consumption and CO_2 production as a function of temperature. Lines drawn to enclose the values obtained were copied from corresponding plots for normal animals whose temperature characteristic was found to be 17,200. Different symbols represent different individuals.

for normal animals. The temperature characteristic of 17,200 then, did not shift as a result of hypophysectomy.

The Immature (red-eft) Stage

It is well known that in the life history of the vermillion-spotted newt the first larval phase, a gilled aquatic form, is succeeded by a coral red larval or red-eft stage (4). This is sometimes referred to as the 'immature land stage' since the reproductive organs are incompletely developed, the gills have been resorbed, and the skin is relatively dry and rough. Mature individuals have a moist, olivaceous skin and are primarily aquatic although they can be kept

for many months on wet filter paper. Adams (5) succeeded in effecting a rapid transformation from the red-eft stage to forms whose skin resembled that of adult animals by means of injections of phyone. I have obtained similar results frequently with sheep pituitary extract (Parke, Davis & Co.), and with frog pituitary implants, but I have been unable to stimulate the accessory sexual tissues even after several months of pituitary administration. In any

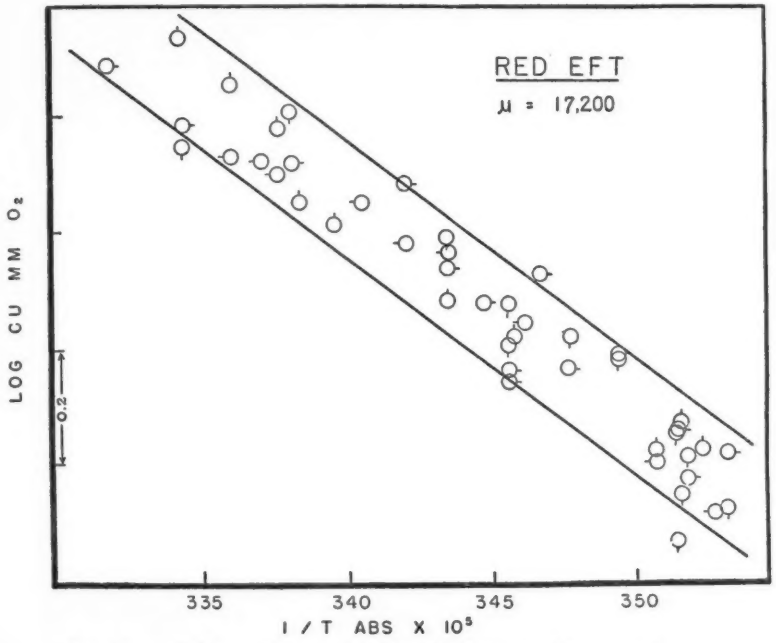

Fig. 2. Immature (red-eft) newts. Arrhenius plot of the rate of O_2 consumption as a function of temperature. Same treatment as given for figure 1.

case, it is obvious that the pituitary is concerned with tissue transformations in the red-eft stage which lead to the assumption of at least most of the adult features. No positive additional information concerning this mechanism was found in the course of these temperature studies. Figure 2, representing the oxygen uptake of 4 typical 'land-phase' individuals again gives a temperature characteristic in the vicinity of 17,200. These animals are negatively phototropic, which probably accounts for the high variability of the data. Although they were shielded from direct light and readings were made only for periods during which the animals remained quiet, all individuals showed long intervals of marked activity.

Respiration Following Thyroidectomy

The thyroids of urodeles are easily located by virtue of their rich capillary supply. The glands can be readily freed from surrounding tissues and pinched off with fine forceps (6).

Using similar criteria for successful operation, temperature range and technic as those given for the hypophysectomy series, the respiration of thyroidectomized animals was measured. An Arrhenius plot of data from 4 animals so treated (fig. 3) showed no significant change in the value of μ.

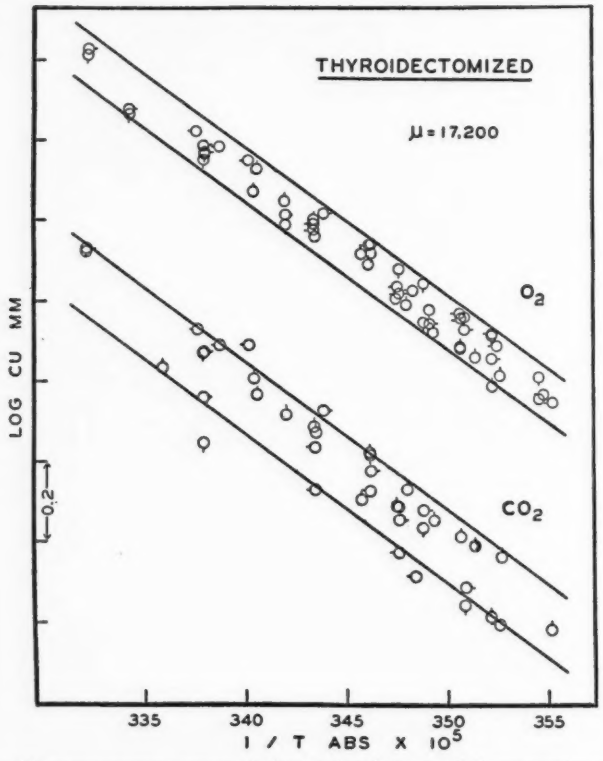

Fig. 3. Thyroidectomized animals. Arrhenius plot of the rate of O_2 consumption and CO_2 production as a function of temperature. Same treatment as given for figure 1.

Respiration Following Pancreatectomy

Using fine forceps, the pancreas of chloretoned newts can be almost completely removed. Great care must be taken to preserve the common bile duct and several large vessels which are surrounded with pancreatic tissue.

Through the cooperation of Miss Hazel Hunt, director of the chemical laboratory of the New England Deaconess Hospital, and Miss McSheffrey of the same institution, quantitative blood sugar determinations were made on the newts used in these experiments. The author is deeply indebted for this assistance.

The technic evolved consisted of delivering the animal's heart out of the pericardial cavity, drying its exterior and draining its contents on filter paper

proven free of reducing substances. The paper was weighed before and after collecting the blood by means of a torsion balance. The micro-method of Folin was used to determine the amount of sugar present in this sample. Normal animals gave values averaging 50 mg. per 100 cc. of blood. Pancreatectomized individuals used in this study showed a minimum value of 150 mg. per 100 cc. of blood.

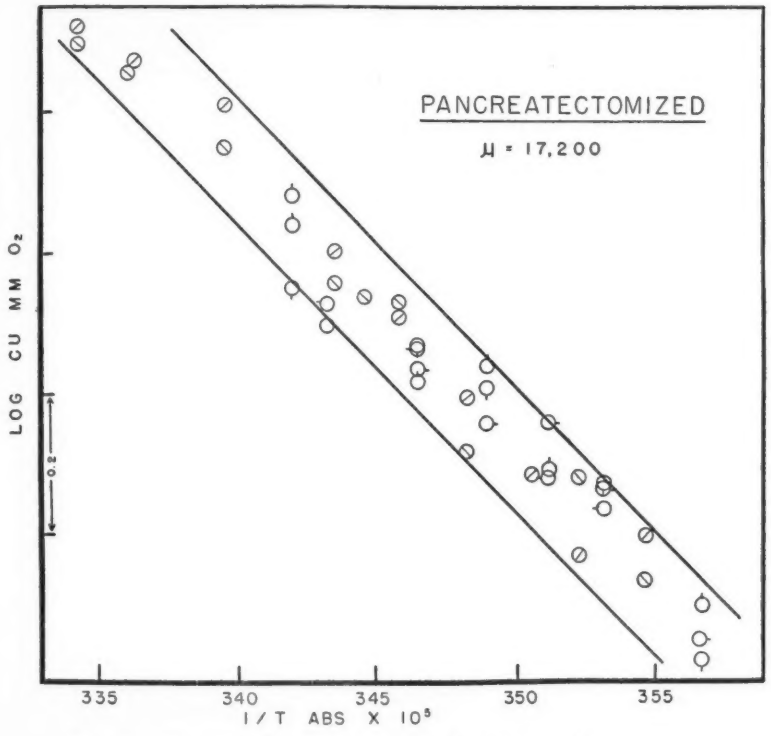

Fig. 4. PANCREATECTOMIZED ANIMALS. Arrhenius plot of the rate of O_2 consumption as a function of temperature. Same treatment as given for figure 1.

Considerable difficulty was encountered in measuring the effect of temperature on such newts. Frequent deaths occurred at the higher temperatures. An Arrhenius plot of data successfully collected under the conditions of the experiment from 5 different individuals (fig. 4) shows no marked deviation from the normal temperature characteristic of 17,200.

DISCUSSION

The temperature characteristic of about 17,000 calories has frequently been found to be associated with biological rates. Crozier (7) made a frequency polygon of the distributions of μ values for some 360 biological rates

and found that 40 of them yielded values of 17,000 ± 500 calories. Hoagland (8) made a frequency plot of 74 temperature characteristics for oxygen consumption and CO_2 production obtained from a variety of studies of cellular respiration described in the literature. Twenty of the 74 values fell between 16,000 and 17,000 calories. The realities of the multimodal distribution of μ values have been demonstrated by the chi-square test and a variety of recent work (8, 9, 10, 11, 12) has been in accordance with the view first advanced by Crozier (13) that the modal μ values correspond to energies of activation of particular catalyzed links in the sequence of reactions constituting cell respiration. The slowest link in such a system would be expected to determine the μ value (14).

It is well known that thyroidectomy and hypophysectomy lower metabolism in animals (15). It is of special interest, therefore, to find that the relation of metabolism to temperature, as measured by the μ value, is unchanged, thus indicating a stability of the relative velocities of the respiratory links which does not entail a shift in chemical pacemaker despite probable overall changes in absolute metabolic rates as a result of hormonal modifications.

SUMMARY

The effect of temperature on the respiratory rate of hypophysectomized, immature (red-eft), thyroidectomized and pancreatectomized newts (*Triturus viridescens*) was studied by means of a modified Warburg respirometer.

On the basis of the data which were obtained it can be said with assurance that in the newt, under the conditions of these experiments, the respiratory rate-temperature relationship (or relative respiratory rate) remains unaffected with the removal of the major endocrine glands (i.e., the μ remains at approximately 17,200). This is interpreted as indicative of a constancy of the chemical pacemaker link despite major endocrine modification.

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STUDIES ON THE INHIBITORY HORMONE OF THE TESTES[1]

II. PREPARATION AND WEIGHT CHANGES IN THE SEX ORGANS OF THE ADULT, MALE, WHITE RAT

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HOSKINS (1) stated that a principle resident in the gonads has an inhibitory effect on the anterior pituitary. The removal of this principle should cause hypertrophy of the pituitary providing the rest of the gonadal secretion remains intact. McCullagh (2) postulated such an inhibitory water-soluble hormone in the germinal epithelium of the testicle. He called this hormone 'inhibin.' The male hormone is produced in the interstitial cells of the testicle and is lipoid-soluble. If the concept of dual hormonology of the testicle is correct, then hyposecretion of the inhibitory hormone should lead to pituitary hypertrophy and resultant hypersecretion of the male hormone. This excess production of the male hormone should then cause hyp ertrophy of the secondary male sex organs.

Martins and Rocha (3) showed that removal of the gonads in rats causes hypertrophy of the pituitary gland. They also demonstrated that the hypertrophied pituitary of a castrated rat causes hypertrophy of the prostate of a normal animal when the animals are in parabiotic union. They were able to prevent this hypertrophy of the prostate in the normal rat by injecting a testicular mush into the castrated partner. McCullagh and Walsh (4) confirmed the above work. Myers, Vidgoff, Hunter and Schade (5, 6) produced atrophy of the prostate in rats by feeding desiccated beef testis. The animals which received the desiccated material for longer periods showed the more striking changes. Lower and his coworkers (7) confirmed this work. McCullagh and Walsh (4) prepared a lipoid-free press-juice of whole beef testes and found that the injection of this material into male rats caused atrophy of the prostate.

In our efforts to isolate the inhibitory hormone of the testes, we first prepared a crude, aqueous extract from bull testes (8) which produced microscopic changes in the sex organs of the male rat and also a decrease in the weight of these organs as compared with the normal animal. In our later preparations of this extract, we made use of a modification of the procedure

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.
[2] Eli Lilly Fellow.

recommended by Deutsch, Eggleton and Eggleton (9), according to which proteins are precipitated with sodium sulfate. This method, in addition to salting out the proteins, removes water and materially concentrates the solution.

Preparation of Extract

Frozen bull testes (9,072 gm.) were obtained from the packing house and left in the refrigerator over night to soften. After the tunics were removed from the glands, the pulp was cut into small pieces and ground in a meat chopper. The resultant mash (7,800 gm.) was warmed to 32° C. in a water bath, anhydrous sodium sulfate (2,290 gm.) added, and the mixture stirred frequently for 5 hours at 32° C. After the mixture stood at room temperature over night, it was again warmed to 32° C., stirred thoroughly, and then pressed by hand. The temperature was kept as constant as possible throughout the procedure to prevent crystallization of sodium sulfate. The thick juice obtained (5,896 gm.) was then filtered under suction through a Büchner funnel fitted with an asbestos mat on filter paper. The filter funnel was equipped with a mechanical stirrer and a jacket through which water at 50° C. flowed. The clear filtrate (2,264 gm.) was cooled to 0° C. and the decahydrate crystals of sodium sulfate which separated out were removed on a filter funnel packed in ice. Two volumes of benzene were added to the filtrate (813 gm.) and the mixture kept in constant agitation for 6 hours by passing air through it. The benzene layer was separated and discarded and the extraction repeated. The aqueous fraction was concentrated by blowing air over it at room temperature. Each cubic centimeter of the final solution represented 25 gm. of the original mash. The final solution also contained 4% sodium sulfate.

EXPERIMENTAL PROCEDURE

The results reported in this paper were obtained in the last of three similar experiments in which the same extract was employed. A fourth series of animals was injected with the extract from which the residual sodium sulfate had been removed by precipitation with alcohol. The results showed that the active principle had been lost. Since a control group of 5 animals given 4% sodium sulfate subcutaneously for 4 to 5 weeks showed no demonstrable changes, the sulfate was not removed from the extract in this work.

White, adult, male rats were chosen from our colony. Ten animals were used as resting controls, while 20 animals received daily subcutaneous injections of the extract for 28 to 35 days. Two of the experimental rats showed infections at the end of the experiment and were discarded. The remaining animals were in good condition as shown by their fur, body weight and body fat. The testicles in all cases were palpated in the scrotum before and after the experiment. All animals were fed on a stock diet of Purina chow food daily and greens twice weekly. The rats were killed with chloroform and autopsied immediately. The sex organs, including the testicles, seminal vesicles, posterior and lateral prostate, were dissected out, weighed on an analytical balance, and then placed in formalin Zenker solution for sectioning. All of the organs were weighed in pairs and the figures in the table represent the weight of the paired organs. The anterior lobe of the prostate was not dissected out but weighed together with the seminal vesicles. The adrenal

glands, thyroid and pituitary were also removed and treated similarly for future work.

RESULTS

Photographs of the sex organs of control and experimental animals were taken. Dissection was made of the complete genital tract, but the pictures

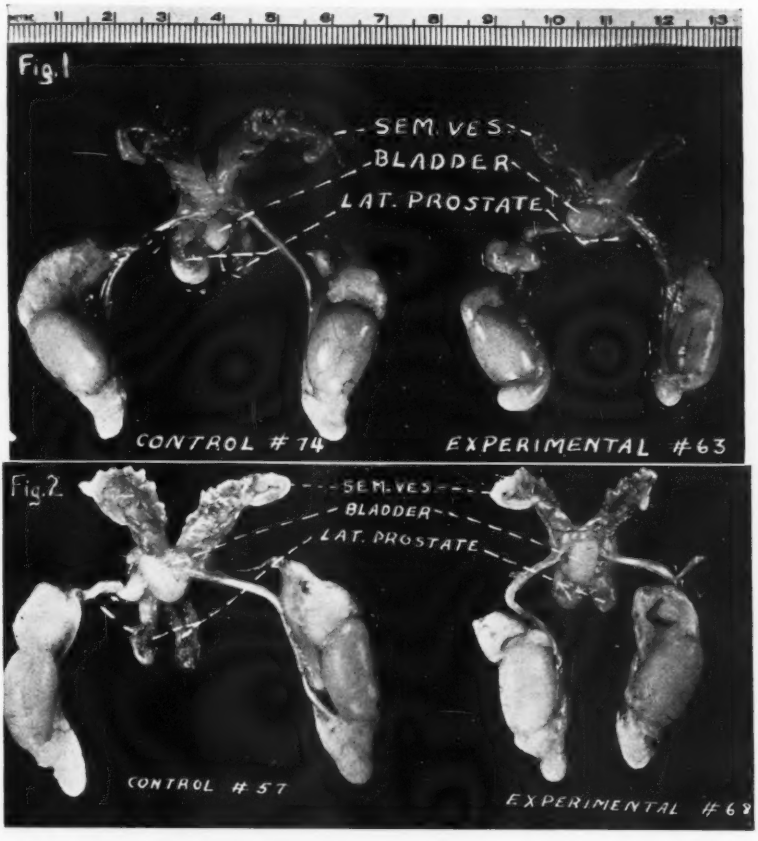

Fig. 1. Anterior view of genital tract of rat. Control rat 74, weight 196 gm.; experimental rat 63, weight 194 gm. received 1 cc. daily subcutaneous injection of the extract for 35 days. Fig. 2. Anterior view of genital tract of rat. Control rat 57, weight 288 gm.; experimental rat 68, weight 280 gm. received 1 cc. daily subcutaneous injection of the extract for 30 days.

show only the testicles, seminal vesicles and the lateral prostatic lobes from the anterior view. Grossly, the sex organs of the experimental animals were reduced in size, especially the prostatic lobes.

Tables 1 and 2 give the reduction in gross weight of the sex organs in experimental animals as compared with those of the control animals. Tables 3

TABLE 1. WEIGHT OF SEX ORGANS OF NORMAL MALE CONTROL RATS (IN GRAMS)

Animal no.	Animal wt.	Posterior prostate	Lateral prostate	Seminal ves. and anterior prostate	Testicle
39	325	0.520	0.590	0.980	3.95
57	288	0.520	0.500	1.230	4.10
58	290	0.228	0.252	0.516	3.92
74	196	0.323	0.408	0.898	3.91
73	246	0.437	0.598	1.137	3.45
75	280	0.540	0.490	0.954	4.35
76	220	0.483	0.540	0.634	3.50
77	244	0.485	0.551	0.963	3.36
78	244	0.386	0.431	0.693	4.43
79	252	0.345	0.552	0.802	4.52

and 4 present ratios of body weight to sex organ weight. This ratio will be referred to as the 'body-organ figure.' Accordingly, the higher this figure the smaller the sex organ weight which it represents.

Prostatic lobes. The prostatic lobes showed more change than the other sex organs. The posterior and lateral lobes seemed to be affected more than the anterior lobes. Grossly, this organ in the experimental animals was smaller and surrounded by more fat than in the controls. A study of the tables shows a reduction in weight of the prostatic lobes in the experimental animals equivalent to one-half to one-third of the weight of the organs in the controls. The average body-organ figures of the control posterior and lateral prostate were 645 and 561 respectively. The figures for the average experimental animal posterior and lateral prostate were 1523 and 1772 in the order named. We have no figures for the anterior lobe of the prostate, because it was weighed with the seminal vesicles. The posterior and lateral lobes in 3

TABLE 2. WEIGHT OF SEX ORGANS OF EXPERIMENTAL MALE RATS (IN GRAMS)

Animal no.	Animal wt.	Posterior prostate	Lateral prostate	Seminal ves. and anterior prostate	Testicle
62	296	0.241	0.280	1.030	3.80
63	194	0.124	0.109	0.191	2.26
64	212	0.122	0.192	0.482	3.36
65	240	0.170	0.140	0.180	2.69
66	200	0.160	0.090	0.230	2.39
67	330	0.530	0.150	0.410	3.75
68	280	0.210	0.140	0.280	3.28
69	312	0.280	0.220	0.660	4.36
26[1]	180			0.100	1.17
25	184	0.055	0.043	0.012	1.84
21[1]	170		0.060	0.122	0.71
20	240	0.175	0.166	0.180	2.80
19[1]	120			0.060	0.74
15	200	0.082	0.185	0.130	3.30
13[1]	160			0.059	0.84
70	260	0.286	0.240	0.882	4.02
71	246	0.211	0.278	0.602	3.38
72	250	0.148	0.172	0.548	3.20

[1] Where there are empty spaces in the table the organs were not weighed. Due to their small size dissection could not be done with any accuracy.

TABLE 3. BODY-ORGAN WEIGHT RATIO OF CONTROL MALE RATS

Animal no.	Animal wt. gm.	Posterior prostate	Lateral prostate	Seminal ves. and anterior prostate	Testicle
39	325	625	550	331	81
57	288	553	576	234	70
58	290	1272	1150	562	74
74	196	607	480	216	50
73	246	563	411	215	71
75	280	518	571	293	64
76	220	455	407	346	63
77	244	502	442	253	72
78	244	632	566	352	55
79	252	730	456	313	56
Average		645	561	312	66

experimental animals (*26*, *19*, and *13*) were too poorly defined for accurate dissection and weighing. In one animal, *21*, the same was true for the posterior lobe although no difficulty was encountered with the lateral lobes.

Seminal vesicles. The seminal vesicles in the experimental animals differed grossly from the normal controls in that they were smaller, had less colloid and were slightly cyanotic. The seminal vesicle weight given in the tables included also, as noted above, the weight of the anterior lobe of the prostate. Much reduction in the weight of these glands in the experimental animals is apparent. The average body-organ figure for the controls was 312 as compared with 1755 for the experimental animals. Most of this weight change was represented by the decrease or absence of seminal fluid in the experimental animals.

Testicle. The testicle also showed weight changes which, however, were

TABLE 4. BODY-ORGAN WEIGHT RATIO OF EXPERIMENTAL MALE RATS

Animal no.	Animal wt. gm.	Posterior prostate	Lateral prostate	Seminal ves. and anterior prostate	Testicle
62	296	1228	1057	287	78
63	194	1564	1778	1015	85
64	212	1737	1104	439	63
65	240	1411	1714	1333	89
66	200	1250	2222	869	83
67	330	666	2222	805	88
68	280	1333	2000	1000	85
69	312	1114	1418	472	71
26	180			1800	153
25	184	3345	4278	15333	100
21	170		2833	1393	239
20	240	1920	1457	1333	87
19	120			2000	152
15	200	2439	1081	1538	601
13	160			2711	190
70	260	909	1083	295	65
71	246	1165	885	410	72
72	250	1689	1453	456	78
Average		1523	1772	1775	104

smaller than observed for the other organs. In some instances the weight of the testicle was greatly reduced in the experimental animals 13, 19, 21, but generally the reduction was not particularly striking. If the change in weight was marked, the testicles appeared cyanotic and felt very soft. In all of such cases the testicles were in the scrotum. The average body-organ figure, contrary to the gross appearance in most of the experimental animals, shows a consistent reduction in testicular weight. This figure was 66 for the controls and 104 for the experimental animals.

SUMMARY

A method for preparing an extract of bull testis which has inhibitory properties is described. The results of subcutaneously administering this extract to adult, male rats are presented.

The posterior and lateral lobes of the prostate showed the most marked decrease in weight. The seminal vesicles were reduced in weight only a little less than the prostatic lobes. The ratio of body weight to organ weight strikingly brings out the difference between experimental and control animals.

The testicles showed a less consistent reduction in weight, but a comparison of the body-weight-organ weight ratios demonstrated that the experimental animals had testicles about one-half the size of those of the controls.

It is a great pleasure to express our appreciation for the suggestions and help given to us throughout this work by Dr. E. S. West, Professor of Biochemistry and Dr. N. A. David, Professor of Pharmacology, University of Oregon Medical School.

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THE EFFECT OF CASTRATION ON BODY WEIGHT AND LENGTH OF THE MALE ALBINO RAT

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BECAUSE OF THE many contradictory reports concerning the effect of castration upon body weight and body length (1), this study was undertaken in an effort to determine the results of gonadal deficiency upon somatic development. This information was particularly important since it was to serve as a basis for comparison in a series of future studies involving hormonal administrations to normal and castrated animals.

For this study 32 male albino rats of Wistar Institute stock were divided into 2 groups of 16 animals each. One group served as test animals, the other comprised litter-mate controls.

Test animals were castrated at 22 days of age. Controls were permitted to retain their testes. All animals were otherwise kept under similar conditions being fed a diet of Purina Dog Chow daily and green vegetables twice weekly. Water was constantly present. Beginning at 26 days of age weekly weights were determined for each animal. On the 80th day of life the animals were anesthetized with ether, their carotids were cut and death was allowed to occur from bleeding. Body lengths, measured between the tip of the snout and anus, were then determined.

All data were tabulated, averaged and probable errors were determined for each group. The calculated mean and its probable error were then compared between the groups and differences noted were considered as probably significant only if the 'significance ratio' was 3 or more (2). In addition, in order to determine whether castration affected body weight and length to the same degree, body weight was calculated according to the formula (3)

$$\text{Body weight}_{(\text{male})} = 10^{\frac{(98.9 \times \text{Body length}) + 13{,}400}{14{,}300}} - 15$$

and the figure obtained was compared with the actual body weight as observed at the time the animals were killed.

RESULTS

It will be noted (table 1) that initial body weights showed no significant difference between the groups while final body weight and length were significantly less by 25.88 gm. and 10.9 mm. respectively in the castrates as compared with the control group. From the graph it may be seen that while the weight curves were practically parallel during the early period of

TABLE 1. COMPARISON OF BODY WEIGHTS AND LENGTHS OF CASTRATED MALE ALBINO RATS WITH THEIR NON-CASTRATE CONTROLS[1]

	Male control	Male castrate	Difference	Significance ratio
Initial body wt. (gm.)	36.00±1.43	38.44±0.91	2.44±1.69	1.44
Final body wt. (gm.)	217.88±3.40	192.0 ±3.85	25.88±5.09	5.11
Final body length (mm.)	205.0 ±1.2	194.1 ±1.3	10.9 ±1.8	6.05

[1] Castration was carried out at 22 days of age, initial weights were determined at 26 days of age and final measurements were taken at 80 days.

observation, they crossed at about 43 days after which time the divergence became progressively more marked.

From table 2, which compares the actual final body weight with the ideal weight calculated for observed final body length, it may be seen that no significant difference exists for the control group, while the castrates possessed final body weights which were significantly greater than the calculated.

DISCUSSION

From the foregoing data it is felt that before castration both test and control groups were in the same body weight and body length class. Body length is included in this statement in spite of the fact that direct measurements were not taken at the outset, for the reason that high correlations normally exist between body length and body weight (3). Direct measurements in young squirming animals would approach the accurate only if anesthesia were used. The significantly lesser final body weight observed in the castrate male is interesting since it stands in contrast to the indefinite 'lean or fat' type previously described by Korenchevsky (4) and the failure to note demonstrable effects on body size by Stotsenberg (5) Moore (6, 7), Overholser (8), Andersen and Kennedy (9). The observation, however, agrees with the reports of Donaldson and Hatai (10), Evans and Simpson (11), Korenchevsky (12), Commins (13) and Lawless (14) who also found an inhibition in body weight of castrated males.

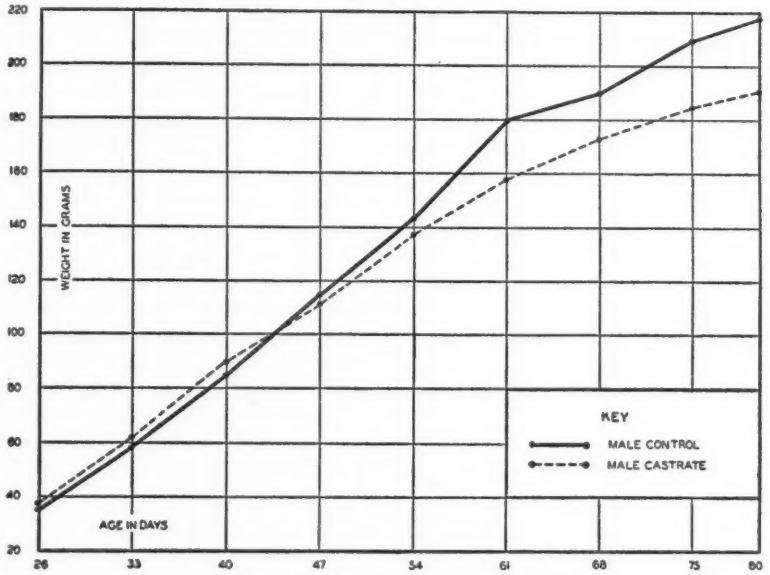
That the effect observed in the rat is species specific is gathered from the observation of Poncet (15) who noted an increase of 8 mm. in length of the capon femur as compared with the cock. In our studies, the skeleton not only failed to become larger, but was actually significantly inhibited in its growth. What is responsible for this somatic weight and growth lag is at present un-

TABLE 2. COMPARISON OF ACTUAL FINAL BODY WEIGHT WITH IDEAL WEIGHT CALCULATED ACCORDING TO FINAL BODY LENGTH

	Body length *mm.*	Actual body wt. *gm.*	Calculated body wt. *gm.*	Difference between calculated and ideal wt.	Significance ratio
Control	205.0±1.2	217.88±3.40	211.4 ±3.40	6.48±4.79	1.35
Castrate	194.1±1.3	192.00±3.85	175.00±3.85	17.00±5.46	3.11

known. The work of Lee and Van Buskirk (16), however, would suggest that it depends upon a metabolic factor.

Whatever is its cause, the effect is produced unequally upon body weight and body length since, as shown in table 2, its inhibitory influence is directed more upon the skeletal system than upon body weight. In other words, while castration in the male rat results in shorter and lighter (in weight) animals, the animal is still relatively heavy for its body length. This observation is at variance with the report of Lawless (14) who found no significant



GRAPH SHOWING AVERAGE WEEKLY WEIGHTS OF CASTRATED MALE RATS AND THEIR CONTROLS

difference by comparing body weight—body length ratios (W/L^3) between his test and control groups. From such calculation, he concluded that "since this ratio is the same in both test and control rats the test rats have about the same build as their controls and their weight is what should be expected, considering their lesser length."

We also noted in our own series, practically similar ratios for the control (0.025), the test (0.026), the calculated control (0.024) and the calculated test (0.024) groups but this similarity was looked upon as being misleading for the following reason. Under normal conditions, it may be in good order to use the cube of any homologous dimension as an index of volume when comparison is to be made between two similarly shaped objects (17). After experimental treatment, however, when one or more of the dimensions (e.g., girth) is altered in one of the groups, it is hardly correct to assume that the two objects are any longer similarly shaped. As a matter of fact, by carrying out

such a procedure one is actually accepting that which one sets out to prove. It is obvious that as an animal becomes relatively fatter, he does not necessarily become longer. Therefore, the denominator (L^3) as used by Lawless fails to reflect any increment of change in any of the three dimensions of the body-volume surrogate which he uses.

On the other hand, it is well known that normally, body weight and body length are highly correlated (3). The high degree of accuracy of the formula of Donaldson is shown in the insignificant difference between calculated and actual body weights of our own control group (table 2). However, the calculated weight of the test group based as it was on the lesser body length of these animals was found to be significantly smaller than their actual weight. In other words, the castrated animals had become significantly heavier than if their weights had been distributed in strict accordance with their inhibited lengths. Although this is the first time so far as we know, that this relatively heavy weight of the castrate has been shown to be statistically significant, Hatai (18) in 1915 had also observed a 3 to 6% increase over the expected weight in gonadectomized rats which were 4% shorter and slightly lighter than their controls.

SUMMARY

Castration of immature white male rats (22 days of age) leads to a suppression of somatic growth as determined by body weight and body length both of which are significantly inhibited. Body length, however, is more affected than body weight so that by 80 days of age the animal has become relatively heavy for its body length.

The authors acknowledge the cooperation of the Ciba Pharmaceutical Products Company, Inc., for partially defraying the expenses of this study.

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A STUDY OF MUSCULAR EFFICIENCY IN RATS INJECTED WITH ANTERIOR PITUITARY GROWTH FACTOR[1]

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IN A PREVIOUS publication, Bartoli, Reed and Struck (1) reported that the anterior pituitary growth factor administered to white rats increased the total nitrogen content of muscle calculated on a dry weight basis and also increased the water content. It seemed of interest to determine whether these changes represent any physiological advantage. It is the purpose of this paper to report the results of an investigation of this point. The literature has been extensively reviewed by Lee (2).

A colony of white rats was divided into two groups. Group 1 served as controls. Group 2 after 28 days of age received daily intraperitoneal injections of the growth factor. Up to 35 days the dose was 2 units per rat, up to 77 days, 3 units, and thereafter 6 units. The diet was the same as described in the earlier paper referred to. The weight curves are shown in figure 1. It should be borne in mind that the groups were progressively reduced in number, and that by accident larger rats were most often selected. Therefore, the differences appear to be less pronounced than would probably be the case were one to carry each group through intact.

Beginning on the 51st day one rat was selected at random from each group, killed by decapitation and the left gastrocnemius was dissected free, except for the attachment to the femur, and mounted for a myogram. The load applied was 50 gm. The muscle was not supported by the afterloading screw of the muscle lever, thus contracting isotonically. Stimulation was done by a condenser discharging at a rate of 80 per minute as described by Schmitt and Schmitt (3). The end point for complete fatigue was taken when, after all visible evidence of activity had ceased, an increase in the intensity of stimulation failed to elicit further contraction. Since all factors remained unchanged throughout, the figures used in the calculations were the actual measurement on the graph instead of the true lift, since these figures could be somewhat more conveniently handled. The reduction ratio of the muscle lever was 15:205.

[1] Part of the expenses of this investigation were borne by a grant from the Nutrition Research Laboratories, and by a grant from the Graduate School Research Fund of the University of Illinois. Parke, Davis & Company made available generous supplies of antuitrin-Growth.

Figure 3 shows typical myograms obtained in such experiments. It must be borne in mind that the selection of animals for each experiment was entirely random. Consequently in some experiments the controls were heavier than the injected animals, in others the weights were identical.

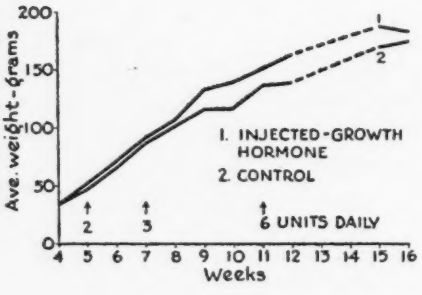

Fig. 1

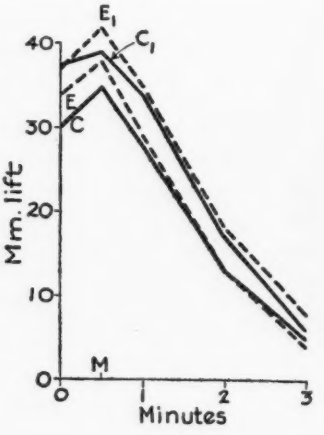

Fig. 2

Fig. 1. Growth graphs of (1), group of female white rats receiving injections of anterior pituitary growth factor; arrows indicate dosages for corresponding periods, (2) Control group. Each group was reduced by one animal daily from the 51st to the 81st day of age and from the 100th day to the 116th day.

Fig. 2. Composite graphs of work performance of isolated rat gastrocnemii stimulated by condenser discharge, 80 per min., at maximum contraction height, and at 1, 2, 3 minutes. *C*, control up to 81 days; C_1, control 100 to 116 days, *E*, E_1, experimental group for corresponding periods. Statistical treatment by Fisher's methods shows significant differences only at point of maximum lift.

In some preliminary experiments on another group of rats where the muscles were stimulated with a tetanizing current it appeared that those from treated rats responded with stronger contractions but fatigued more rapidly; also that with progress in treatment the differences would become more pronounced. It seems probable that had the plan of investigation been made differently, the results might have been more striking. Actually there was very little change in the relative responses of the muscles after 89 days of treatment as compared to the initial experiments. Therefore, it would have been better had all of the rats been kept alive until the end of the period. Also, the criticism may be made that isolated mammalian muscle undergoes rapid physiological deterioration and, therefore, the muscles should have been perfused in situ during the period of stimulation. However, it was felt that if any significant differences in efficiency existed, these would be accentuated by subjecting the muscle to more drastic treatment. The standard period from decapitation to the first stimulation was 3 minutes in all experiments.

It was impossible to keep the load adapted to the growth of the rats, so the same load was used through the 81st day. An interval of 19 days then

elapsed during which treatment was continued but no myograms were made. It therefore seemed advisable to increase the load to 62 gm. and to treat the data in two sections.

The adoption of criteria for comparison was a matter of considerable

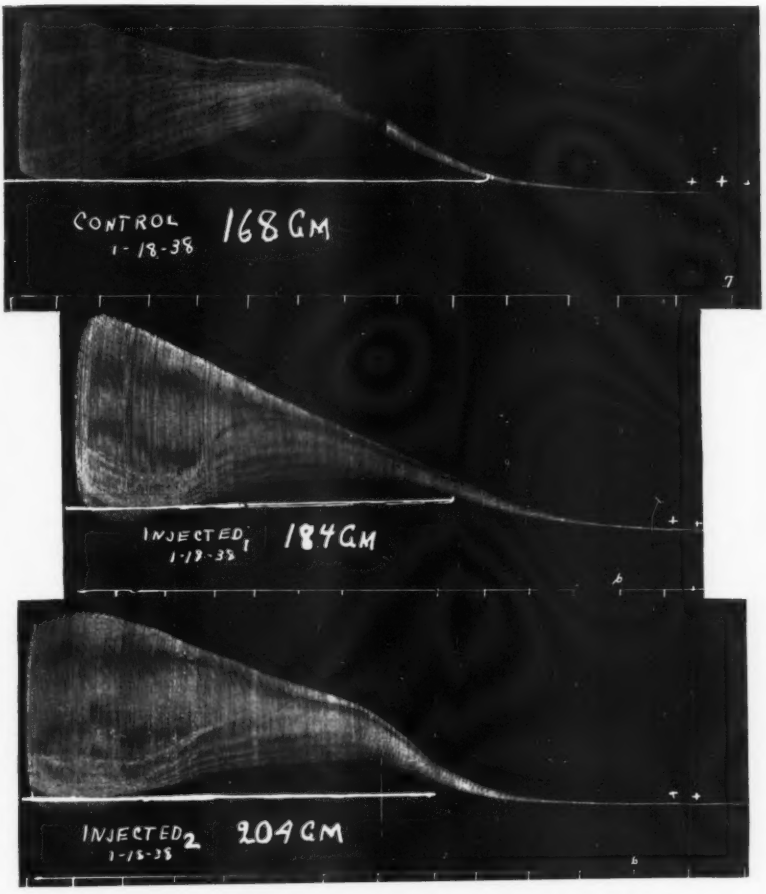

Fig. 3. Myograms of isolated rat gastrocnemii stimulated by condenser discharge, 80 per min. + strength of stimulus increased. Base line indicates initial length of muscle.

difficulty. The maximum amplitude of contraction found at any time is not entirely satisfactory for the reason that not all of the muscles showed treppe. When treppe appeared the maximum occurred at varying intervals after the initial stimulation but always within the first minute. The duration of activity was likewise unsatisfactory for the reason that after development of a sufficient degree of fatigue so that the graphs were only a few millimeters high this state often existed for long periods. Obviously, there was very

little energy involvement so that it appeared unjustifiable to compare such a graph with one in which there was a very rapid loss of efficiency to the point of complete fatigue.

Finally the graph in figure 2 was constructed by taking the measurements of the initial contraction, at 1, 2, 3 minutes, and at the maximum only when treppe occurred; and also the time at which the amplitude was reduced to exactly one-half of the initial contraction. Using Fisher's formula[2] (4), these composite graphs were constructed. The lower pair (C, E), represent all the experiments up to 81 days, the upper pair (C_1, E_1) those from 100 days on. According to Fisher's tables of statistical probability there are significant differences apparent only in the maximal contractions. So far as these experiments are concerned there is, then, a significant increase in the power of the growth hormone treated muscles to contract for the brief period of maximal contraction in treppe but otherwise there is no physiological advantage. In fact, if one takes into account that, while individual rats in the treated group were lighter than their controls, the average weight of the group was significantly greater than for the control group and, therefore, it might be expected that muscular efficiency would be greater due to exercise. The average time required to reduce the initial length of the graph to one-half was, in the first section, 113 seconds for the control group and 111 seconds for the experimental group. In the second section the averages were, respectively, 115 and 118 seconds. Obviously these differences are not significant.

These results would appear to be at variance with the observations of muscular weakness in gigantism. But when one considers that these experiments represent the threshold of effect of the hormone and that, in spite of greater average size of the injected rats and therefore greater weight of muscular tissues (a few accurate weighings showed that the weights of gastrocnemii are roughly proportional to body weight), there was no physiological advantage, it is comprehensible that no great disparity is apparent. It is then predictable that with larger doses and more prolonged administration, the muscles of treated rats would show great deficiency.

SUMMARY

Myograms taken on gastrocnemii muscles of female white rats treated with anterior pituitary growth extract, in spite of greater average size of the treated rats, showed no physiological advantage over those from untreated controls.

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[2] $T = \frac{X_1 - X_2}{S} \sqrt{\frac{N_1 \times N_2}{N_1 + N_2}}$

$X_1 - X_2$ = Difference between means of two groups.

$S = \sqrt{\frac{\Sigma + \Sigma}{N_1 + N_2}}$ N = number in group; Σ = sum of squares of differences of individual values from mean; T = probability reference factor to be used with Fisher's Tables.

SYMPOSIUM ON ENDOCRINES*

CHEMISTRY OF THE ADRENAL CORTICAL HORMONE

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ACTIVE CHEMICAL work on the adrenal cortex dates from about 1930 when Swingle and Pfiffner (1) and Hartman and Brownell (2) announced the preparation of extracts capable of maintaining the life of adrenalectomized animals. The work has been carried on, in the main, by three groups, Reichstein and his associates at Zurich, Wintersteiner and Pfiffner (3) at Columbia, and Kendall, Mason and associates (4–9) at The Mayo Foundation. After the isolation of several compounds Wintersteiner and Pfiffner gave up their work, so that most of the information has been obtained in the other two laboratories. Reichstein (10–21) has made brilliant contributions in the isolation and determination of the structure of a large number of compounds. We have also contributed to the determination of structures but have centered our attention on those substances which are active physiologically, particularly on that substance, as yet unisolated, which possesses the highest degree of activity.

A brief outline of the extraction and purification procedures will serve to orient some of the later discussion. The three laboratories mentioned have used alcohol, which is essentially the procedure of Swingle and Pfiffner for at least the first stage of the extraction. After distribution between petroleum ether and 70% alcohol and two distributions between ether and water, Reichstein (10, 13) resorted to the use of Girard's reagent for the separation of ketonic from nonketonic material. He thus succeeded in separating 7 or 8 compounds. Later (17), acetylation of the residues and adsorption analysis permitted him to raise the number of compounds isolated to 22. We have avoided as much as possible chemical means of separation since invariably most of the physiologic activity could not be recovered after such treatment. Distributions between water and chloroform and between water and benzene have enabled us to isolate 9 compounds and application of acetylation and adsorption analysis to the residues has yielded two more.

Although 6 compounds with cortin-like activity have been isolated in

* The following seven papers were read in a symposium sponsored by the Division of Medicinal Chemistry of the American Chemical Society, at Baltimore, April 4, 1939. Chairman: Dr. Walter H. Hartung. Secretary: Dr. Russel J. Fosbinder.

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crystalline form, it is our experience that they account for only a minor portion of the activity of the original extract. The part of the extract that produces the major portion of the activity passes from water into benzene and from benzene into water with very little loss so that even after 15 distributions 50% of the activity remains.

The amazing array of compounds which Reichstein has succeeded in isolating from the adrenal gland includes a good share of the possible combi-

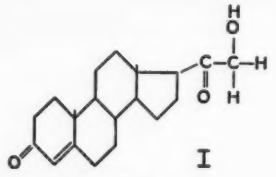

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Fig. 1.

nations of 3, 4 and 5 oxygen atoms in the progestane nucleus limited to substitutions at C-3, 11, 17, 20 and 21. Even progesterone and allo-pregnanolone with two oxygen atoms are among them (22). However, this discussion will be limited to those compounds which have demonstrable ability to maintain the life of an adrenalectomized animal.

First, we shall consider the most active crystalline compound, desoxycorticosterone (I) (fig. 1) which has been isolated only very recently from an extract of the adrenal cortex by Reichstein (16) but which was earlier prepared by him (18) from stigmasterol to serve as a model. Its structure is therefore known to be that of 21-hydroxyprogesterone. Its high activity lends support to the assignment of the α, β-unsaturated ketone groups of the other compounds to the same position as in progesterone. The exact position of this group in the other compounds, however, has not been proved with finality.

The next most active compound is one which we have labeled compound B (II) and which Reichstein (23) has named corticosterone. It has a com-

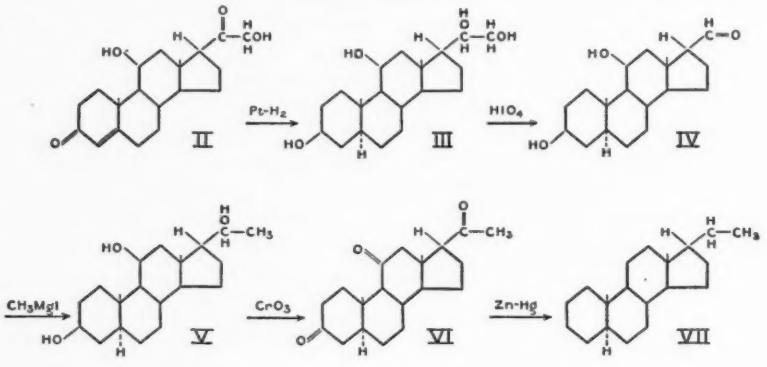

Fig. 2.

panion which we call compound A which has the same order of activity. Steiger and Reichstein (19) furnished the proof that corticosterone has a steroid skeleton by the reactions shown in figure 2. Corticosterone (II) was first completely hydrogenated with platinum and hydrogen. Oxidation of

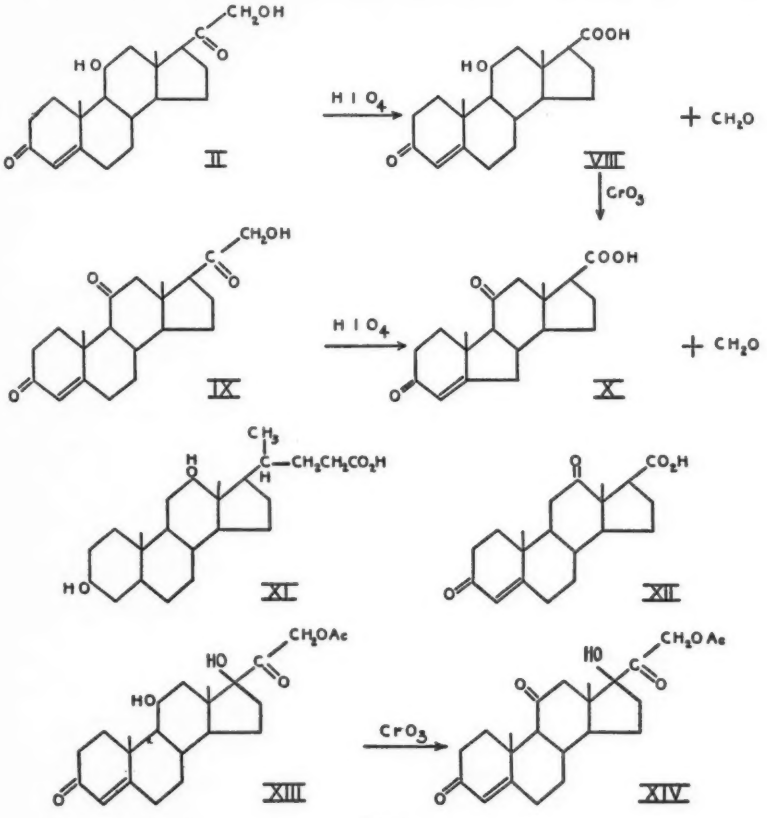

Fig. 3.

the product (III) with periodic acid gave the aldehyde IV which was converted to the triol V with methyl magnesium iodide. Oxidation with chromic acid produced the triketone VI which was reduced by Clemmensen's method to allo-pregnane (VII).

We (7) were able to determine the nature and location of the oxygen atoms. The presence of an α, β-unsaturated ketone group was established by means of the absorption spectrum. The ketone group was assigned to C-3 and the ethylenic bond to the 4-5 position by analogy with progesterone and other compounds of similar nature. As further support for this assumption hydrogenation of this group produced a saturated alcohol which was precipitated by digitonin.

The presence of the α-ketol group assigned to C-17 was established with the aid of periodic acid which cleaves a primary α-ketol to an acid and formaldehyde (fig. 3). Thus, 3 of the 4 oxygen atoms were placed and their functions established. The oxidation of compounds A (IX) and B (II) with periodic acid gave two acids which differed in composition by two hydrogen atoms. Since the acid (VIII) from compound B could be converted into the acid (X) derived from compound A by oxidation with chromic acid, the fourth oxygen atom of compound B was shown to be a hydroxyl and that of compound A a ketone group. Attempts to determine the nature of this oxygen atom by other means had failed since it is exceedingly inert. Acid chlorides with pyridine and even phosphorus pentachloride failed to react with the hydroxyl group in this position. Carbonyl reagents such as hydroxylamine could not be made to react with the corresponding ketone group. However, the Grignard reagent would add to this carbonyl group and also reacted with the hydroxyl group in this series of compounds.

In view of the inert nature of this oxygen atom it has been assigned to carbon 11 since a similar inert oxygen of sarmentogenin has also been assigned to this position and since all other positions except 12 appear to be excluded on the basis of activity toward various reagents and their relations to the known substituents. It should be emphasized, however, that the position of this oxygen atom has not been conclusively established. Hoehn and I (5) have eliminated position 12 as a possibility by comparison of the acid (X) obtained from corticosterone with the acid of analogous structure (XII) obtained by the degradation of desoxycholic acid (XI) (fig. 3). Since X and XII had different properties, position 12 for the location of the inert oxygen was excluded provided the assignment of the α, β-unsaturated ketone group of X to the position shown is correct. Its location in any other position is highly improbable.

The work of Tschesche and Bohle (24) indicated that digoxigenin possesses a hydroxyl group at carbon 11 just as sarmentogenin apparently does but with the opposite configuration. In an attempt to settle the position of the inert oxygen atom of the adrenal compounds Steiger and Reichstein (20) degraded digoxigenin to the corresponding Δ^4-diketoetiocholenic acid. Hoehn and I (6) were able to show, however, by comparison of two of the acids derived by Steiger and Reichstein from digoxigenin with the corresponding ones derived from desoxycholic acid that they are identical and that therefore the relatively inert oxygen atom of digoxigenin occupies carbon 12. The only possibility for comparison of adrenal compounds with a known natural product is sarmentogenin which is very difficult to obtain.

Other compounds with the least, but still definite, activity are our compounds E and F(8) which are Reichstein's compounds Fa and M. Reichstein (14) showed their relationship by oxidation of the 21-acetate of compound F (XIII) to the acetate of compound E (XIV) (fig. 3).

Our compound E (XV) which was isolated at about the same time by Wintersteiner and Pfiffner (3) and known as their compound F, was the first crystalline compound for which cortin-like activity was demonstrated. This

claim was doubted by many because of the large amount required to maintain an adrenalectomized dog. However, not only has the claim been substantiated with respect to the ability to maintain adrenalectomized animals but the

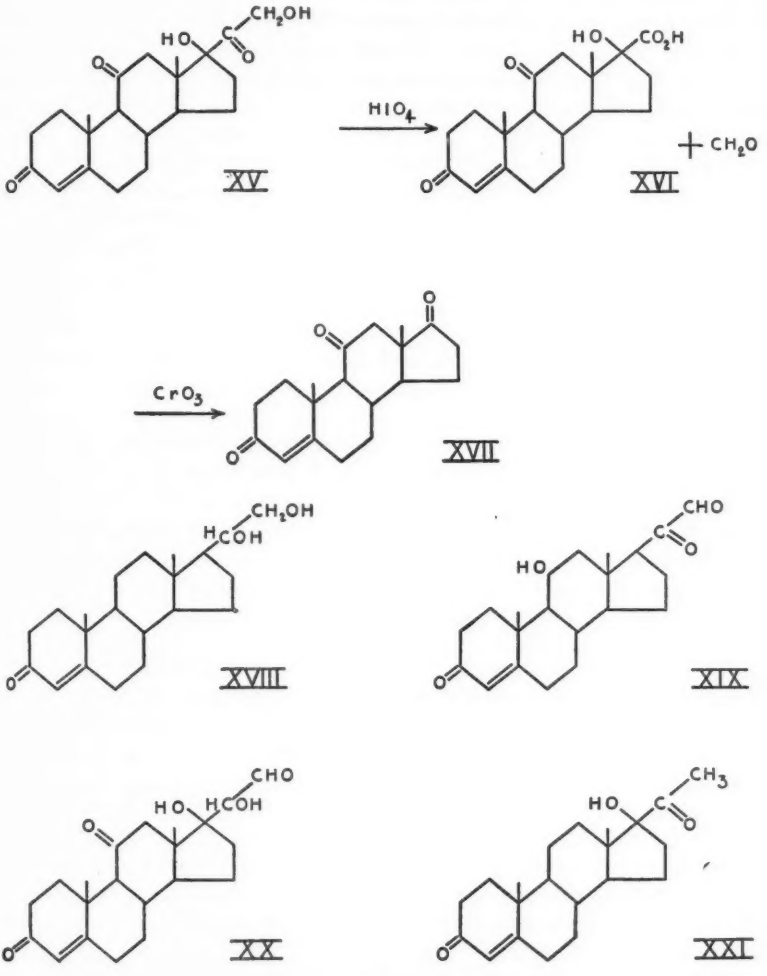

Fig. 4.

compound has been shown by Long[1] to have diabetogenic activity in depancreatized-adrenalectomized rats.

Following the demonstration by Reichstein (11, 12) that a substance which he called adrenosterone, and ketonic degradation products of another

[1] Reported at the Fiftieth Annual Meeting of the American Physiological Society, Baltimore, Maryland, March 30-April 2, 1938.

compound are capable of producing comb growth in the capon, it was found that a ketone (later shown to be adrenosterone, XVII) prepared by oxidation of compound E also possesses this property (9). Thus was furnished the first definite evidence relating cortin activity with steroid structure, although Reichstein (12) had previously suggested such a relation. Later Reichstein (15) correlated all the members of the $C_{21}O_5$ series, to which compound E belongs, through their ketone degradation products and furnished the proof that these are steroid compounds by conversion of one of the degradation products to androsterone.

The presence of an α, β-unsaturated ketone group and the α-ketol side chain was demonstrated as before (fig. 4) (9). Further oxidation of the acid (XVI) obtained from compound E (XV) with periodic acid yielded a ketone (XVII) with one less carbon atom. This result established the presence of a hydroxyl group adjacent to the carboxyl group. These reactions placed 4 of the 5 oxygen atoms. Analysis with the Grignard reagent indicated that the fifth oxygen atom is present as a hydroxyl group. Later (8), however, it was found to be a ketone group which does not add the Grignard reagent as it does in the $C_{21}O_4$ series but enolizes to yield an active hydrogen atom.

There are two features which distinguish the compounds that have physiologic activity. They are the α, β-unsaturated ketone group and the α-ketol side chain. We (7) have shown that reduction of the ethylenic bond alone will destroy this activity. It seems probable that the α-ketol side chain is necessary for highest activity but it is not absolutely essential since progesterone, which Gaunt and Hays (25) have recently shown to have the ability to maintain the lives of adrenalectomized ferrets in amounts of 2 to 5 mg. daily, has a very low order of activity. The activity of compounds with the two features mentioned decreases from desoxycorticosterone to compound E with the increasing number of oxygen atoms. In spite of these relations our experience with the fractionation of these compounds and the concentration of the compound of greatest activity has indicated that the most highly active compound which is yet to be isolated may be a rather highly hydroxylated compound. This active compound goes from benzene or ether into water with considerable ease and the final active concentrate is relatively soluble in water. It is difficult to correlate this observation with the high physiologic acitivity of desoxycorticosterone and corticosterone which remain preferentially in the benzene solutions when they are extracted with water.

It may be noted that conversion of the hydroxyl group at C_{11} of corticosterone to a ketone group has little if any effect on the activity although complete removal of this oxygen atom increases the activity 6 times. The same relation holds for the activities of compounds F and E.

When considering structure and activity it is necessary to take into account the different effects on various species and the nature of the physiologic test. It has been found in this laboratory that desoxycorticosterone (I) is approximately six times as active as corticosterone (II) in adrenalectomized

dogs. For a 20 kg. dog the daily requirement of desoxycorticosterone is about 0.3 mg. given in a single dose; for corticosterone the daily requirement is about 2 mg. while for compound E (XV) it is 7 to 10 mg. However, Ingle (26) found that in his rat test, which is a fatigue test on adrenalectomized rats, compound E and corticosterone are equally effective. The minimal dose in both cases is 0.125 mg. but the minimal effective dose of desoxycorticosterone is 2 mg. It has further been found that with dogs desoxycorticosterone acetate is 2 to 3 times as active as desoxycorticosterone. Ingle (26) was unable to distinguish any difference in his rat test. These figures are to be considered as comparative only since many physiologic variables make it difficult to compare values found in one laboratory with those found in another.

If we assume, as is most likely, that the very active compound still unisolated must have the α, β-unsaturated ketone grouping, the possibilities seem rather limited for enhancement of the activity by changes in the known substances, particularly in view of the greater activity of the less highly oxygenated compounds. Consideration of the side chain indicates several possible changes. Reichstein (21) has tested one of these possibilities by preparation of the compound (XVIII) analogous to desoxycorticosterone but having a hydroxyl group instead of a ketone group at C_{20}. He was unable to demonstrate any activity in this compound.

We have considered another possibility, that of an aldehyde group at C_{21}. We have prepared such a compound (XIX) from corticosterone by oxidation with selenium oxide and found that the activity was diminished. It cannot be stated definitely that the compound has any activity since we did not isolate it completely from unchanged corticosterone but certainly the activity was not enhanced. The presence of this compound was demonstrated by preparation and analysis of its quinoxaline. We have also attempted to prepare the dihydroxyaldehyde (XX) by rearrangement of the α-ketol group of compound E in alkaline medium and in pyridine at 100° C. but without success (fig. 4). The material remained unchanged, underwent a more deep-seated rearrangement or formed an amorphous gum. A few hours' treatment of compound E with a saturated solution of calcium hydroxide had little effect; most of the substance was recovered unchanged. More prolonged treatment caused a rearrangement of the side chain to an acid and also effected cleavage of the side chain from part of the material. (4) Even tenth-normal sodium hydroxide had no effect on corticosterone in the cold. Although pyridine and heat are reported to rearrange dihydroxy acetone to glyceric aldehyde this treatment for 20 hours had no effect on compound E except for a small amount of gum formation.

Recently Ruzicka and Meldahl (27) have described the preparation of 17-hydroxyprogesterone (XXI) which is reported to have about one-eighth of the activity of desoxycorticosterone or about the same activity as corticosterone.

The very active concentrate which has been mentioned represents a concentration from about 20 gm. of solids derived from 300 pounds of

glands to 0.5 gm. and contains about 50% of the activity of the original crude extract. It is active on dogs in amounts of 1–2γ per kg. of body weight daily. The activity appears to be more stable to heat than in the case of crude extracts yet the activity may disappear with disconcerting abruptness under other apparently mild conditions. For example, evaporation of a chloroform solution to dryness in a stream of carbon dioxide has at times resulted in almost complete loss of activity. This loss was traced to the development of a trace of acid in the chloroform which apparently caused a dehydration of the material when the solution was taken to dryness. In any event, mineral acids and dehydrating agents, such as copper sulfate, in anhydrous media have the same effect. The product is then exceedingly insoluble in water and consists of a mixture of polymers which have an average molecular weight of about three times that of the original material. The latter has a molecular weight of approximately 360 as determined by osmotic pressure measurements with a thermocouple. Analysis of this material shows a composition close to that calculated for $C_{21}H_{28}O_5$ or $C_{21}H_{30}O_6$.

Examination of the highly active material which is still undoubtedly a mixture shows the presence of carbonyl groups. It is almost completely bound by Girard's reagent and about 90% is released on acidification to pH 1. It was hoped that oxidation with periodic acid would yield information of value but the results were indeterminate. The product from one preparation was about equally divided between acid and nonacid and the yield of formaldehyde was 60 to 70% of 1 mol. based on a molecular weight of 360. Aside from traces of known acids and ketones the oxidation products could not be crystallized.

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THE RÔLE OF THE ADRENAL GLANDS IN THE ANIMAL ECONOMY

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THE ADRENAL GLANDS are tiny inconspicuous organs which lie buried in the peri-renal fat adjacent to the kidneys. The combined weight of the two glands in the human adult is about 13 grams. Despite their small size the glands are of vital importance to the organism for their removal leads to a complete breakdown in the normal functioning of many organs and tissues of the body and results in death in a short period of time. The existence of the glands was first noted by Eustachius in the sixteenth century but modern studies on their significance date from 1855 when Addison first described the clinical disorder which results from destruction of the glands by disease (1).

The adrenal glands are compound organs derived from two separate types of tissues. The cortical portion of the gland is glandular in structure while the medullary portion is derived from a common progenitor with the sympathetic nervous system. In the lower animals (e.g., the fishes), these two tissues remain separated as distinct organs. The significance of their union in the mammal is still a matter of speculation. The product of secretion of the adrenal medulla is epinephrine first isolated by Abel in 1901 as the *N*-benzoyl derivative. Aldrich and Takamine subsequently isolated the free base whose remarkable pharmacological properties have rendered it a most valuable therapeutic agent. The synthesis of epinephrine by Dakin and Stolz in 1904 established its structure as 2-methylamino-β-hydroxy-β-(3,4-dihydroxyphenyl) ethane. Synthetic epinephrine is optically inactive. To obtain the levorotary isomer, which is about 15 times as active as the racemic compound, advantage is taken of the fact that the *d*-tartaric acid salt of *dl*-epinephrine is soluble in methyl alcohol while the levo-isomer is not. Synthetic *d*-epinephrine has all the properties of the natural product of the gland and competes with the latter as a source of the epinephrine of commerce (1).

Despite its importance as a therapeutic agent, the function of epinephrine in the animal economy is still debated. Pharmacologically, epinephrine exerts activities which simulate those elicited by stimulation of the sympathetic nervous system. It is therefore designated as a sympathicomimetic substance. Many extraneous stimuli cause the discharge of epinephrine from the adrenal; e.g., physical agents, such as heat or cold, chemical stimuli, such as strychnine, nicotine and insulin. The adrenal medulla may, however, be removed from the organism without resulting in any apparent functional

disability of the organism. Cannon (2) has, therefore, advanced the theory that the adrenal medulla subserves an emergency function in the organism. According to this theory, epinephrine exerts no function in the normal resting organism but aids in the maintenance of homeostasis in emergencies.

It is the cortical portion of the gland which is vital to the organism and hence most recent work has been devoted to the elucidation of the rôle of the cortex in the animal economy. In 1931, Swingle and Pfiffner (3) described the preparation of an extract of the adrenals which was capable of maintaining the life of the adrenalectomized animal. Subsequent workers modified the method of preparation of this extract which is at present available as the purified aqueous extract for parenteral injection or as an adsorbate on charcoal or crude concentrate for oral administration. These products are utilized clinically for conditions due to a deficiency of the hormone in the body.

As a result of the chemical studies of Kendall, Reichstein, Wintersteiner, and their coworkers, over twenty crystalline derivatives have been isolated from adrenal extracts. These compounds have been demonstrated to be steroid derivatives which are closely related to the hormones derived from the reproductive system. Although several of these crystalline derivatives derived from glandular extracts have been claimed to exert some biological activity it is extremely doubtful if any of them represents the true hormone. The most potent of these compounds thus far described was prepared first by Steiger and Reichstein (4) and subsequently isolated from glandular extracts. This compound, known as desoxycorticosterone, is capable of maintaining the life of adrenalectomized animals when given in moderate doses. One milligram per day suffices to maintain an adrenalectomized rat in good health. Beneficent effects have also been described to follow the administration of this material, in the form of its acetate, in Addison's disease (5). Desoxycorticosterone is a derivative of progesterone (a crystalline compound derived from extracts of the corpus luteum) in which an hydroxyl group is substituted in the methyl group of the side chain in the 17-position. Progesterone itself manifests some ability to replace the cortical hormone in the organism but requires exceedingly large doses (over 4 mg. per day in the rat). It is possible that tissues other than that of the adrenal are capable of converting certain related steroids to the adrenal cortical hormone or else that other compounds may actually replace the true hormone in performing its normal function.

It is very improbable that desoxycorticosterone represents the natural hormone for the amounts of this substance necessary to maintain life in adrenalectomized animals is many times that of even relatively crude concentrates derived from glandular extracts. The author has isolated a crystalline compound having an empirical formula $C_{19-20}H_{28-30}O_5$ which exerts 100 times the biological activity of desoxycorticosterone. Nevertheless, the difficulty and expense of preparing extracts from adrenal glands render the synthetic production of desoxycorticosterone of greatest importance to medicinal chemistry. It is, moreover, within the realm of possibility that

other derivatives of this compound, which more closely approach the hormone in composition, may be prepared in the future.

Many unsuccessful attempts have been made to define the exact mode of action of the adrenal cortical hormone. It is indeed questionable if any of the hormones exert a single specific activity. At least none has been demonstrated for any of the known hormones. The hormones resemble the vitamins (which have been called the exogenous hormones) in exerting manifold activities. As in the case of the vitamins, it is probable that some very basic chemical reaction in the organism is dependent upon the presence of the hormones. Hence, their absence in the body will result in manifestations of disordered function in many tissues and organs. This has been shown to be true in the case of the adrenal hormone. Its deprivation results, for example, in a disability of the kidney to perform its normal function (7). Consequently vitally important substances such as water and sodium chloride which are normally spared for the organism, are lost through the kidney in adrenal insufficiency, while waste products, such as potassium and urea, normally excreted, accumulate in the blood. The liver in adrenal insufficiency likewise shows a deficiency as regards its normal function in carbohydrate and cholesterol metabolism. The muscles are unable to contract normally and manifest marked asthenia. Other organs and tissues show similar evidences of deficiency (1).

The adrenals are involved in several diseased conditions. Loss of their function, usually by tuberculosis or atrophy, results in Addison's disease. No clinical condition has been described which may be attributed to overproduction of cortin. However, certain tumors of the cortex give rise to pathological masculinization. This process is due presumably to the production by the gland of androgenic substances. When this pathological process occurs in uterine life, pseudo-masculine hermaphroditism results. Occurring in later life it may induce the development of secondary masculine characters in the female. This assumption of an androgenic function by the adrenal may be looked upon either as due to the inclusion of embryonic androgenic tissue in the course of development or to a perversion of the normal metabolism of the gland. It is conceivable that the diseased organ might produce an androgen instead of the chemically related cortical hormone.

The chromaphil cells of the adrenal medulla also give rise to certain tumors. However, there is no definite clinical syndrome attributable to medullary insufficiency. Certain chromaphil cell tumors, presumably by liberating an excess of epinephrine into the circulation, may give rise to paroxysmal hypertension.

Besides the two hormones already described—epinephrine and cortin—a number of other compounds have been isolated from the adrenal. Ascorbic acid (vitamin C), for example, occurs in a high concentration in the gland and was first isolated in crystalline form by Szent-Györgyi (8) from this source. However, it is doubtful if the presence of vitamin C or of the other compounds derived from the gland play a fundamental rôle in the economy of the gland. Their presence in such relatively high concentration in the

gland may be explained as incidental to the physico-chemical conditions determining the distribution of substances in the organism.

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THE CHEMISTRY OF THE SEX HORMONES

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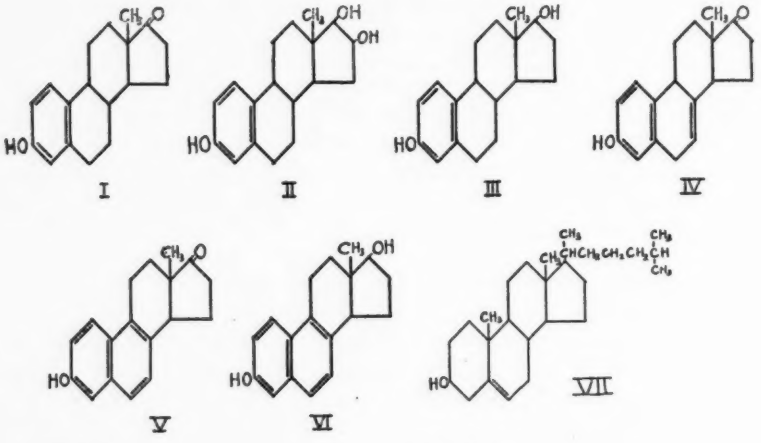
THERE HAVE BEEN few fields of organic chemistry in which the developments have been so rapid and dramatic as in the field of the sex hormones. Within the space of a decade we have seen not only the isolation of the various female and male sex hormones but also the elucidation of the molecular structure of these complicated compounds and their preparation in the laboratory from inactive substances. In making these achievements possible certain contributions were of prime importance. For the isolation of a hormone a reliable bioassay procedure is necessary, and it was the development of the Allen-Doisy vaginal smear method for assay of the estrogens which made possible the isolation of the interesting series of these hormones which we now know. This method is based on the fact that the course of the estrual cycle of the rat or mouse is characterized by changes in the nature of the vaginal epithelium. The course of the cycle may be followed by microscopic examination of stained smears of the vaginal contents. The vaginal smear characteristic of the ovariectomized rat or mouse consists only of leucocytes. If estrogenic material is administered to such an animal the smear changes to one consisting entirely of squamous non-nucleated epithelial cells. This occurs in the course of 36 to 48 hours.

Another contribution which was of utmost importance was the discovery by Aschheim and Zondek (1) of the high concentration of estrogenic material in human pregnancy urine. All previously discovered sources either contained relatively insignificant amounts of the active material or gave extracts which were extremely troublesome to purify.

With the development of a satisfactory bioassay procedure and the discovery of a cheap and convenient source it was to be expected that the isolation would be accomplished in due time. Preparation of the first female sex hormone, theelin or estrone, (I) in a pure crystalline condition was reported in 1929 by Doisy, Veler and Thayer (2) and shortly thereafter by Butenandt (3) and by Laqueur and his collaborators (4). It has also been isolated from a variety of other sources, namely, mare pregnancy urine, stallion urine, male human urine, placenta and palm kernel oil. The international unit of estrogenic activity has been established as the specific activity of 0.1 microgram of crystalline estrone.

In the year following the isolation of theelin from human pregnancy

urine a second estrogen was isolated from this source independently by Marrian (5) and by Doisy and his coworkers (6). This substance (II) is known as theelol or estriol and has also been obtained in a pure crystalline condition from placenta and from female willow catkins. Both theelin ($C_{18}H_{22}O_2$) and theelol ($C_{18}H_{24}O_3$) were found to possess a phenolic hydroxyl group. The second oxygen atom of theelin was found to be ketonic while the two other oxygen atoms of theelol were shown to be in the form of secondary hydroxyl groups. By heating theelol with potassium acid sulfate Butenandt and Hildebrandt (7) found that it could be converted to theelin with loss of one molecule of water.



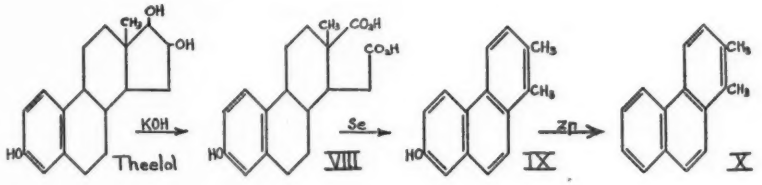
I Theelin
II Theelol
III Dihydrotheelin
IV Equilin
V Equilenin
VI Dihydroequilenin
VII Cholesterol

The first estrogenic hormone to be obtained from the ovary was reported from this laboratory in 1935 (8). It was found to be a dihydro derivative of theelin (III) having the composition $C_{18}H_{24}O_2$ and it is the most potent estrogenic substance thus far isolated from natural sources. It is also found in mare pregnancy urine where it is accompanied by a less potent stereoisomer. Due to the low concentration (sow ovaries contain one part of dihydrotheelin in 160,000,000) its isolation presented some difficulties and it was necessary to process 4 tons of ovaries to obtain 11 mg. of a crystalline derivative from which the hormone could be regenerated. Since the compound had previously been prepared in the laboratory by the reduction of theelin, this amount sufficed for identification of the hormone. It is from 2 to 8 times as active as theelin depending upon the method of assay. The C_{17} epimer which is found in mare pregnancy urine is only about one-third as active as theelin.

In addition to the estrogens already mentioned as occurring in the urine

of pregnant mares, several other active compounds have been isolated from this source by Girard et al. (9, 10). Three of them, equilin, (IV), equilenin (V) and dihydroequilenin (VI) are well characterized; the existence of a fourth, hippulin, is doubtful.

In 1928 Windaus and Wieland were jointly awarded the Nobel Prize in Chemistry for their brilliant researches in the field of sterols and bile acids. Out of their work there was evolved their structural formula for cholesterol which gained such wide acceptance. However, there were certain facts which could not be reconciled with the formula and a careful consideration of these led Rosenheim and King (11) in 1932 to suggest a new formula in which the ring system was that of a partially reduced cyclopentenophenanthrene. The essential correctness of this formula (VII) was quickly appreciated and when it was shown that the estrogens possessed



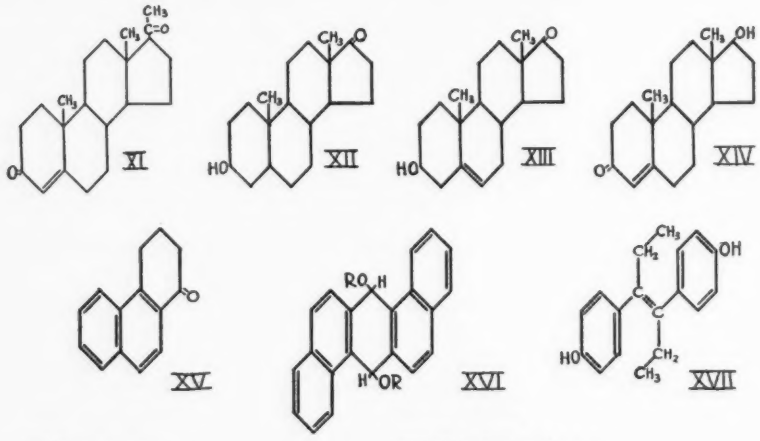
the sterol ring system the elucidation of their structure proceeded with rapidity.

It was shown in this laboratory (12) and independently by Marrian and Haslewood (13) that when theelol was fused with potassium hydroxide a 5-membered ring was opened between adjacent carbon atoms with the formation of a dibasic acid (VIII). It was found by Butenandt, Weidlich and Thompson (14) that when this acid was heated with selenium it underwent decarboxylation and dehydrogenation to a dimethylphenanthrol (IX) which on distillation with zinc dust gave 1,2-dimethylphenanthrene (X) which was identified by comparison with a synthetic specimen. These experiments served to indicate clearly the relation of the hormones to the sterols.

Structurally the estrogenic hormones are closely related. They are all phenols containing the steroid ring system. In all of them ring A is aromatic and the phenolic hydroxyl is at position 3. Ring B shows progressive degrees of unsaturation, having an ethylene linkage in equilin and forming a naphthalene nucleus in equilenin and dihydroequilenin. A second functional group, which may be a carbonyl or a secondary alcohol group is always present at position 17. Theelol contains a third hydroxyl group at position 16.

Upon rupture of the graafian follicle and extrusion of the ovum a tissue is formed which is known as the corpus luteum and which performs the function of preparing the uterus for implantation of the fertilized ovum and maintaining it in a suitable condition for development of the embryo. The initial proliferation of the uterine mucosa is stimulated by the estrogenic

hormones but the hormone present in the corpus luteum causes a much greater proliferation of the endometrium so that when the fertilized ovum reaches the uterus implantation readily takes place and gestation begins. The most reliable and widely used method for evaluating the progestational activity is known as the Corner-Allen test. Mature rabbits are ovariectomized 18 hours after mating (to induce ovulation) and are then given daily subcutaneous injections of the substance to be assayed. Injections are given for 5 days and on the sixth day the animal is killed and the uterus examined histologically to determine the extent of proliferation. In the Clauberg test, which is more convenient but somewhat less reliable, immature rabbits are used so that castration is not necessary. This necessitates a preliminary stimulation of the uterus by injections of estrogen, after which the administration of a corpus luteum preparation will cause proliferation.



XI Progesterone
XII Androsterone
XIII Dehydroisoandrosterone
XIV Testosterone

The isolation of the hormone of the corpus luteum was announced almost simultaneously in 1934 by four groups of workers—Butenandt (15); Slotta, Ruschig and Fels (16); Allen and Wintersteiner (17); and Hartmann and Wettstein (18). By common consent it was named progesterone (XI). It occurs in two distinct crystalline modifications which, however, have the same biological activity. The international unit of progestational activity has been established as the specific activity of 1.0 mg. of progesterone. Several methods have been described for the preparation of progesterone in the laboratory.

The bioassay procedure which has proved most useful for estimation of androgenic or male sex hormone activity is known as the comb test. It depends upon stimulation of comb growth in the capon resulting upon administration of androgenic material. For estimation of the effect the comb may be

measured directly or the silhouette of the comb may be photographed and the area measured by means of a planimeter.

The first naturally occurring androgen to be obtained in a pure crystalline form was isolated by Butenandt and Tscherning (19) from male urine in 1931. They named the new hormone androsterone (XII). The international unit of androgenic activity has been established as the specific activity of 100 micrograms of crystalline androsterone. Three years later a second compound possessing male hormone activity was isolated from the same source by the same group of investigators (20). Since it contains two less hydrogen atoms than androsterone it was called dehydroandrosterone (XIII). Subsequent work showed that it differs structurally from androsterone in another respect, namely, the configuration at carbon atom 3. It is therefore more correctly called dehydroisoandrosterone.

In 1935 Laqueur and his collaborators (21) isolated the third natural androgen. The source was testis tissue and the name suggested was testosterone (XIV). The structural relation of the androgens to the sterols was indicated by the experiments of Ruzicka (22) in which he succeeced in converting cholesterol into androsterone. Testosterone is the most potent androgen known. It also has some female sex hormone activity. It causes premature opening of the vagina in young rats and mice, and it also has some progestational activity although the dosage required is relatively large. Progesterone however has neither estrogenic nor androgenic activity. Progesterone shows a very high degree of specificity. Only a very few other compounds are known to have any progestational activity and these only in relatively high dosages. In contrast a great many artificial androgens and estrogens are known. The first of the latter to be discovered by Cook et. al. (23) was 1-keto-1,2,3,4-tetrahydrophenanthrene (XV) which is active at a dosage of 100 mg. for the castrated mouse. An interesting series of active compounds was found in the 9,10-dihydroxy-9,10-dialkyl-9,10-dihydro-1,2,5,6 dibenzanthracenes (XVI) (24). One synthetic compound is worthy of special note in this connection. Dodds, Robinson and their collaborators (25) studied the estrogenic activity of the 4,4′ dihydroxy-α, β-dialkyl stilbenes (dialkyl 'stilbestrols') and they found that the diethyl derivative (XVII) was considerably more active than estrone and at least as active as estradiol. It appears to possess all of the physiological properties characteristic of estrone. Whether it may produce any undesirable results in the human organism remains to be determined.

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CLINICAL EMPLOYMENT OF THE FEMALE SEX HORMONES

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THE BIOLOGICAL CHEMIST has played a part no less important than that of the clinician, the anatomist and the physiologist in the remarkable advances which have been made in the field of endocrinology during the past quarter century or so. The combined efforts of all these groups have, indeed, furnished a fine example of teamwork in a field in which the need of such correlation of effort is especially great. While the most outstanding accomplishments of recent years are to be credited to the laboratory rather than to the clinic, the rôle of the clinician has not been an unimportant one.

The mere search for truth is in itself always laudable, but most of us will agree with the late Lord Moynihan that the goal of investigation in the medical sciences should be in some way related to the relief of human disease. The genuine contributions which have already been made to our understanding of many endocrine disorders, and, to a considerably less extent, to the treatment of these disorders, is sufficient proof that this greatest of objectives has not been lost sight of. Certainly this statement applies to one of the largest fields of endocrinology, that revolving around the study of the female reproductive cycle, and from the standpoint of the clinician, represented by the large group of functional disorders in women.

It has seemed to me that clinicians are developing a sort of inferiority complex in the study of endocrine problems, so awed are they by the brilliant contributions which have been coming from the laboratory, and so dependent have they become upon the laboratory workers for the ammunition which they so sorely need in their own clinical work. Indeed, it would almost seem as if the clinic has become only a proving-ground where the results of laboratory work on animals are put to the human test, sometimes justifiably and rationally, sometimes otherwise. And yet there are certain advantages which the clinician enjoys over the laboratory worker. From the standpoint of clinical application, the final test of endocrinological investigations must be based upon observations on the human patient, and the latter may even justifiably be used as an experimental animal in at least a limited way. Quite obviously the clinician cannot hypophysectomize or castrate his patients with the gay abandon of the laboratory experimentalist, nor can his observations always be so well controlled and precise. And yet, if he is seriously interested in endocrine problems, he can make worthwhile contributions through careful clinical research, including such aids as histological studies, blood and urine hormone determinations, and endometrial biopsies.

No one will be startled by the admission that there are few clinicians who are deeply versed in the intricacies of biochemistry, and I for one cannot expect to speak to you in your own language today. It must be the other

way around, and my participation as the clinician in this symposium must in all discretion be devoted to a survey of a few of our clinical problems, in an effort to show how we have tried to apply to their management the advances which have been following each other so rapidly that we poor clinicians might well be forgiven some bewilderment and confusion in our effort to keep up with the frequently somewhat erratic scientific bandwagon.

The fact that there must be two separate ovarian hormones, with quite different effects upon the endometrium, had been assumed by gynecologists long before the two principles were actually isolated, chiefly on the basis of correlative histological studies on the ovary and endometrium. Now that both hormones are so readily available for clinical employment, there is certainly no justification for the employment of the various forms of desiccated ovarian and corpus luteum substance which enjoyed such wide vogue up to a few years ago. In retrospect, our old discussions as to the value or lack of value of these substances now seem amusing or pathetic, depending upon the viewpoint. Here, then, is a contribution of outstanding importance, for now we may at least be reasonably sure of the physiological potency of the preparations with which we work.

Far different is the status of affairs with regard to the gonadotropic hormones of the anterior pituitary lobe, for as yet we know nothing of their chemical structure, and it has not been possible to prepare extracts of undoubted efficacy in the human patient. And yet each year new additions are made to our knowledge, so that sooner or later biochemists will solve this problem for us as they have that of the ovarian hormones. It seems reasonable to look forward to this as offering hope for a more intelligent and effective treatment than is now possible in the case of amenorrhea.

Much is written about the etiology and the classification of cases in this disorder, and certainly such studies are important. The fact remains, however, that the prevailing treatment of this condition is notoriously unsatisfactory, our armamentarium consisting essentially in the administration of thyroid extract, estrogenic substances perhaps supplemented by progesterone, and such gonadotropic substances as are available, including those prepared from the pituitary itself, from the serum of the pregnant mare or from castrate urine. While the combined or sequential use of estrogen and progesterone does seem to substitute for a deficient ovarian function more nearly than estrogenic therapy alone, I have not, after a rather extensive personal experience, been able to convince myself that the percentage of failures is appreciably less than when estrogenic therapy alone is employed.

It may seem unorthodox even to discuss estrogenic therapy in amenorrhea, because of the accepted fact that it produces no stimulating effect upon the ovary itself, and that excessive dosage may actually inhibit ovarian function through the medium of the pituitary. And yet it does seem that in some cases a quantitative deficiency in the patient's own estrogen may thus be filled and an approximately normal interplay between the hypophysis and ovary initiated. Furthermore, it is logical to believe that in some cases an undeveloped endometrium may be sufficiently sensitized by the administered estrogen to become responsive to the patient's own hormones.

This labored defense of the present widespread vogue of estrogenic ther-

apy in endocrinopathic amenorrhea exposes the fact that in this field clinicians are still floundering, their only excuse being that, having patients to treat, they must try to do the best they can with present day knowledge and preparations. They will gladly throw such methods overboard and clasp to their bosoms any more rational or more effective measures with which our laboratory colleagues may supply us in the future. In view of the unsatisfactory status of endocrine therapy in amenorrhea, it is small wonder that other methods, such as x-ray treatment, have been resorted to, with just as little rationale, but according to some enthusiasts, with much more frequent success. That, however, is not within the scope of the present discussion.

I need hardly add that what has been said above as to estrogenic therapy in amenorrhea should not be taken as applying to all cases. For example, the so-called 'polyhormonal' cases, characterized by persistent follicle-hormone excess and perhaps persistent hyperplasia of the endometrium, would have to be excepted. This, however, is not a very large group, and in my experience the amenorrhea is generally punctuated, though perhaps at long intervals, with bleeding of normal or excessive amount. Even in the numerous cases of primarily hypophyseal origin, estrogenic therapy may usually be logically combined with the very ineffective gonadotropic therapy as yet available.

As regards the opposite quantitative disorder of menstruation, functional bleeding, I think it may be stated that endocrine therapy, in those cases in which it is indicated, has been much more frequently successful than in amenorrhea. The most common type of functional bleeding, though not by any means the only one, is at bottom a disorder of ovulation, and it is quite possible that when we know more about the hormonal mechanism of ovulation and of some means of promoting ovulation in non-ovulating women, we can correct this type of functional bleeding more fundamentally than we can at present. The recent studies of Davis and Koff on the purported effectiveness of pregnant mare serum preparations in promoting ovulation in women do not appear convincing, as I have discussed in a recent paper, and the more accurately controllable studies of Hartman with the same substance upon a large series of non-ovulating monkeys make its value seem very questionable indeed.

The hormone principles which have been most widely employed in the treatment of functional bleeding are the anterior pituitary-like hormones of pregnancy urine, progesterone, and more recently testosterone. As to the first of these we know little or nothing as to the mechanism involved, though we do know that these substances produce no such luteinization in the human ovary as they do in the ovaries of certain experimental animals. We know scarcely more concerning the effects of progesterone in the comparatively small doses commonly employed, doses too small to bring about any noteworthy histological effect upon the endometrium. Here we are brought into contact with the perennial discussion of a 'bleeding-factor' in menstruation, theoretically possible, but as yet unsupported by any sound evidence.

Just at present it is testosterone which is in the limelight as regards the treatment of functional bleeding. Whether its benefit comes from the fact that its effects in some respects are like those of progesterone, or whether

these effects are to be attributed to an inhibition of follicle activity through the agency of the pituitary, is still not certain. Thus far its clinical effects seem to be more frequently successful than those of the other preparations I have mentioned, and this has been my own experience. However, the method is still new, and at least one disadvantage has already appeared, through the report of cases in which hirsutism, deepening of the voice, and even enlargement of the clitoris have followed the use of the substance. I have seen no such cases myself, possibly because I have employed smaller dosages than those given in the above mentioned cases. Moreover, I would be inclined to think that such manifestations would be temporary, though even so they would constitute a genuine drawback to this method of treatment. A wider experience with this plan of organotherapy is necessary before it can be properly evaluated.

A report of successful results in the treatment of functional bleeding has recently been made by Hamblen, his method consisting in the cyclic administration of estrogen and progesterone, or of progesterone alone, after initial checking of the bleeding attack by estrogen or curettage. Twelve patients received in all 46 series of these treatments, with good results in 45 of the latter. The theoretical basis for the treatment does not seem altogether sound, embracing as it does a number of unproved assumptions, but the good results reported would seem to justify a trial of this plan by others, in view of the limited helpfulness of other methods of organotherapy.

As regards the treatment of the vasomotor symptoms of the menopause, the solidification of opinion as to the efficacy of estrogenic therapy is clearly to be explained by the undoubted physiological potency of the preparations now so readily available. Contrast this with the confusion and uncertainty of ovarian therapy before the isolation of the estrogenic hormone, when it consisted of the administration of tablets, capsules, or powders of whole ovary, corpus luteum, or ovarian residue. Many gynecologists were completely skeptical of results, but some were enthusiastic, and this uncertainty dragged along for nearly a quarter of a century. In retrospect it would seem quite certain that any good effects of such therapy must have been explainable on the basis of its psychic effect upon the patients. On the other hand there is now almost complete unanimity of opinion as to the efficacy of estrogenic therapy in making the menopausal woman's lot much less unpleasant than it might otherwise be in a not inconsiderable proportion of cases.

It seems to be well established that the underlying cause of the characteristic subjective symptoms of the menopause is a cessation of ovarian function, but there is reason to believe that the immediate factor is the increased gonadotropic activity of the pituitary which is so characteristically associated with absence or marked deficiency of estrogen production. Be that as it may, there is a sound rationale in the administration of estrogens in the minority of menopausal cases in which the symptoms are sufficiently troublesome to constitute a real problem. I have in a recent paper discussed this problem at some length, emphasizing the importance in the management of such cases of other measures in addition to organotherapy. In considering the matter of dosages to be employed, one cannot be arbitrary, especially

when one considers that the quantitative endocrine relationships between the hypophysis and the gonads quite certainly exhibit wide individual variations, so that, in general, the dosage should be that required to give satisfactory relief to the patient.

The introduction of testosterone into the therapeutic field has led to its employment by many in the treatment of menopausal symptoms. Its chief advantage is stated to be the avoidance of the uterine hyperplasia and bleeding which may follow large doses of estrogen. The latter sequelae, however, are relatively rare and always transitory, and the relief afforded to the patient has not, in my experience, been any more frequent or pronounced than that obtainable from the estrogens. The latter, moreover, are somewhat less costly in corresponding doses, so that my own preference in most cases is still for estrogenic therapy. I have already alluded to the recent reports of cases in which the employment of testosterone has brought about hirsutism and other masculinization manifestations.

Finally, only a brief reference need be made to hormonal therapy in the treatment of primary dysmenorrhea, because we know so little as to the etiology of this disorder. The tendency of recent years is to link it up with a heightened irritability and contractility of the uterine musculature, but it must be admitted that there is no clear proof that this is the real cause. The majority of investigators believe that estrogen is the normal stimulant of the uterine musculature and progesterone the inhibitant, but there are not a few dissenters from this view. It is therefore not surprising that, while many employ in treatment such substances as proluton, or the luteinizing principle of pregnancy urine, others advocate estrogenic therapy, with results by neither plan which could be called constant or convincing. The same thing may be said with reference to the use of testosterone, although here the published reports are still too few to furnish a basis for worthwhile evaluation.

There are two interesting clinical observations which seem to me worth stressing in any discussion of the hormonal relationships of primary dysmenorrhea. I have been impressed with the fact that in a very large proportion of cases, this disorder does not date from the actual inauguration of the function, but that it begins somewhat later, in some cases only a few months, in some several years. This at least suggests that the beginning of ovulation and progesterone production may be the important factor, and that an imbalance of the two ovarian hormones may be more important than an actual absence of progesterone. Again, dysmenorrhea is characteristically absent in cases of functional bleeding, in which again there is a relative excess of estrogenic hormone and an absence of progesterone.

These clinical facts, at any rate, must be squared with any hormonal theory of dysmenorrhea to make it tenable. The recent studies upon the importance of the vascular apparatus of menstruation, with the reasonable certainty that this is under the control of the ovarian hormones, makes it pertinent to inquire whether abnormal vascular response due to endocrine imbalance of the type predicated above may not play the important rôle in the etiology of primary dysmenorrhea. Such a question is easily posed, but it cannot be answered until we know more as to the exact nature of the

vascular changes and the mechanism of their control by the ovarian hormones.

Many other applications of endocrinology to gynecology might be discussed if time permitted. For example, an established part of the treatment of habitual or repeated abortion, if one may judge from the literature, is the administration of progesterone, because of the importance of this hormone in the early stages of pregnancy, and because of its supposed deficiency in cases of the type mentioned. Treatment of this sort is not easily adaptable to scientific control, but published reports would seem to justify its continuance. Still another clear-cut application of biological knowledge to clinical practice is in the undeniably effective treatment of the gonorrheal vulvovaginitis of children with estrogenic substances, and, while not always quite so impressive, the estrogenic therapy of senile vaginitis. Still another application, based upon the inhibitory effect of large doses of estrogenic substance upon the pituitary, is to be noted in the frequently successful estrogenic therapy of the definitely menstrual headaches encountered so frequently in gynecological practice. And still others might be mentioned.

The laboratory worker is apt to look with contempt, perhaps righteous, at the rather loose scientific methods so painfully apparent in many clinics in the investigation of endocrine problems and the evaluation of organotherapy. On the other hand, clinicians may at times be irritated with the clinical aloofness of many laboratory students of reproductive physiology. There is a measure of justification in both these attitudes, but they are certainly not irreconcilable. In so far as the clinician is concerned, his first duty is to acquire an intelligent working knowledge of reproductive physiology, and to apply this, in as nearly a scientific way as possible, to the study and management of the endocrine problems encountered in his daily work.

This paper has been concerned with a discussion of some gynecological problems which illustrate the practical utilization of both laboratory and clinical studies in endocrinology. In the clinical employment of the ovarian hormones a tremendous advance has occurred in that we now have available preparations of known composition and undoubted physiological potency. This has removed at least one great factor of uncertainty in the evaluation of the clinical results of ovarian therapy. Our efforts at gonadotropic therapy are still woefully inadequate because of the lack of preparations of undoubted efficacy in the human patient, and also our ignorance concerning many aspects of the reciprocal endocrine relationships between the hypophysis and ovary. A good example of this is seen in the incompleteness of our knowledge as to the hormonal mechanism of ovulation, and as to any means of promoting ovulation in non-ovulating women. Similar scientific gaps in other problems are inevitably reflected in even larger gaps in the clinical fields. In filling in these deficiencies, the clinician can make at least a modest contribution, though the larger responsibility must rather inevitably be shouldered by the laboratory worker.

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THE RELATION OF THE ANTERIOR PITUITARY TO SEX AND METABOLISM[1]

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THE PHYSIOLOGICAL importance of the anterior pituitary for the normal function of the body in regard to sex development and metabolism has been established by the investigations of various research workers (1, 2, 15, 30). The pharmacodynamic function of the anterior pituitary has been studied either by extirpation or implantation of the gland or by the injection of various pituitary preparations which, however, were generally rather impure. The number of separate distinct hormones secreted by the anterior pituitary is, unfortunately, still a matter of opinion and dispute.

Pituitary research, a field where simplification is urgently needed, has gone through two distinct phases, the first of which may be considered as exploratory or descriptive in character. During this first period a perplexing number of supposedly separate active principles, produced and secreted by this gland, has been postulated. Unfortunately, some investigators in this field have been inclined to claim the discovery of an additional distinct pituitary principle when observing a new physiological response of a crude pituitary extract rather than attributing a multiplicity of physiological actions to one and the same factor. Thus a large number of separate specific principles has been claimed to be produced and secreted by the anterior pituitary.

TABLE 1. SEPARATE ANTERIOR PITUITARY HORMONES POSTULATED

A—Gonadotropic hormones
- 1) Follicle stimulating
- 2) Luteinizing
- 3) Interstitial cell stimulating
- 4) Antagonist
- 5) Synergist
- 6) Activator

B—Thyrotropic hormone
C—Adrenotropic hormone
D—Lactogenic hormone
E—Growth hormone
F—Metabolic hormones
- 1) Diabetogenic
- 2) Ketogenic
- 3) Pancreatropic
- 4) Glycotropic
- 5) Glycostatic

From this confusing situation, pituitary research has entered now into the second phase which may be considered quantitative in nature. Efforts are

[1] This paper represents a résumé of a lecture presented by the senior author (H. J.) at the Meeting of the American Chemical Society in Baltimore, April 1939. The bibliography is admittedly incomplete. For a review and bibliography of the earlier work on this subject the reader is referred to a recent monograph by van Dyke (1) and to the "Symposia on Quantitative Biology" (2).

being made to compare on a quantitative basis physiological effects which have been ascribed to separate pituitary principles. In some instances it has already been found that several physiological effects can be attributed to only one pituitary principle, the response depending on the dose level and the route of administration.

It has also been realized, in recent years, that the other endocrine glands through their own secretion products exert a regulatory influence on the pituitary. This interrelationship is illustrated, for instance, by the changes which take place in the histological picture and hormone content of the anterior pituitary after castration and by the fact that these changes can be prevented by the administration of sex hormones to the castrated animal (3). Histological changes in the pituitary have also been observed upon removal of the thyroids and adrenals (3). Numerous attempts are also being made to achieve further chemical separation and purification of the various pituitary factors.

It is not possible to discuss in this paper all the manifold physiological effects supposedly exercised by the anterior pituitary; reference will be made only to the more important ones. It may be said here that recent findings indicate that the anterior pituitary does not secrete as many different distinct principles as was assumed at one time.

In this connection it may also be opportune to remark that, on studying the literature, one is rather disturbed by the frequent lack of detailed information concerning the chemical procedure employed by different laboratories in the preparation of the various anterior pituitary factors. The dose injected is often expressed in terms of the original wet or dried pituitary material instead of physiological units. It is obvious that such a method of standardization is inaccurate and may lead to wrong conclusions. It also makes it difficult to compare similar results reported from different laboratories. We feel that it is essential to determine the biological response of the various AP principles on a quantitative physiological basis.

Relation of the Anterior Pituitary to Sex

The investigation of the functional interrelationship of the anterior pituitary to the gonads has resulted in the accumulation of a large amount of data which unfortunately do not permit any definite conclusions. This may be attributed to the fact that results are often contradictory and therefore allow a considerable freedom of interpretation. Again, the subject of this interrelationship has further been complicated by the postulation of several different gonadotropic principles which are supposed to be necessary for the normal development of the mammalian gonads (see table 1).

Investigations which we have carried out during 1938 at the Institute of Experimental Biology, University of California, in collaboration with Dr. H. M. Evans and Dr. M. E. Simpson have shown that the gonadotropic activity of sheep pituitary can be separated into two components, *a*) a follicle stimulating (FSH) and *b*) an interstitial cell stimulating (ICSH) principle (4, 5).

Combination experiments (4, 5) with these purified FSH and ICSH prep-

arations carried out on a quantitative basis in hypophysectomized female rats[2] have shown that *synergism* (activation, augmentation of ovarian weight) and luteinization occur at certain dose levels of both factors under the following conditions: *a*) when the two components are mixed in vitro and injected subcutaneously; *b*) when the two components are injected subcutaneously at different sites; *c*) when a preliminary injection of FSH is followed by ICSH, both given subcutaneously. On the other hand, *antagonism* (decrease of the FSH response) can be demonstrated if: *a*) the two factors are combined in vitro and given intraperitoneally; *b*) ICSH is given intraperitoneally concurrently with FSH given subcutaneously. On the basis of these findings, we feel, therefore, that the assumption of a separate synergist or activator and of a separate luteinizing and interstitial cell stimulating hormone and antagonist is not justified at present. The primary development of the ovary is apparently controlled by the proper physiological coordination of two hypophyseal hormones, namely the follicle stimulating hormone and the interstitial cell stimulating hormone (luteinizing hormone, LH).[3] In the male, the germinal epithelium is stimulated by the FSH and the interstitial tissue (Leydig cells) by the ICSH; both principles also act synergistically to each other in the male (6).

The augmentative effect between FSH and ICSH, consisting of an increase in ovarian weight and formation of corpora lutea, is apparently due to a specific synergism between the two hypophyseal principles. This is in contrast to the non-specific augmentation observed by Saunders and Cole (7) and Maxwell (8) on addition of certain inert substances (casein, zinc sulfate) to gonadotropic pituitary preparations. The latter effect can probably be attributed to a decrease in the rate of absorption of the active principles.

The antagonistic action of hypophyseal ICSH can also be demonstrated in normal and hypophysectomized rats, against the gonadotropic activity present in pregnant mare serum.[4] On the other hand, it has been observed that the effect of pregnant mare serum in regard to ovary weight can be diminished when given intraperitoneally in combination with either purified FSH preparations (5), thyroxine or estrogenic compounds (9). The mechanism and possible physiological significance of these various antagonistic effects are being investigated. The intricate rôle which the sex hormones play in

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[2] In these quantitative biological studies we adopted the following physiological units: *Follicle stimulating hormone* (FSH): one rat unit represents the minimal total amount which administered subcutaneously ½ daily over a 3-day period into hypophysectomized rats (26 to 28 days old at operation, 6 to 8 days post-operative interval before injection) causes resumption of follicular growth, as evidenced at autopsy 72 hours after onset of injection. *Interstitial cell stimulating hormone* (ICSH): one rat unit is the minimal total amount which administered intraperitoneally ½ daily over a 3-day period into hypophysectomized rats (26 to 28 days old at operation, 6 to 8 days post-operative interval before injection) causes repair of the deficient interstitial tissue of the ovary, as evidenced at autopsy 72 hours after onset of injection.

[3] In regard to the LH or ICSH nomenclature, the term ICSH seems to us preferable since a single biological effect, namely the repair of the deficient interstitial tissue of the ovary or testis of the hypophysectomized rat can be accurately defined and determined by itself without involving combination with the FSH factor.

[4] Preliminary fractionation experiments, carried out at the Institute of Experimental Biology, University of California, in collaboration with Dr. H. M. Evans and Dr. M. E. Simpson, have shown that the gonadotropic activity of pregnant mare serum is due to the presence of two gonadotropic factors, FSH and ICSH. We feel that the ICSH factor in pregnant mare serum differs from the hypophyseal ICSH and from prolan (chorionic gonadotropin) in certain chemical and biological properties.

the interrelationship between the pituitary and the gonads will not be discussed here.

A biological comparison of the hypophyseal ICSH (LH) with the gonadotropic pregnancy urine principle (prolan or 'chorionic gonadotrophin')[5] reveals certain differences both in normal and hypophysectomized animals (5). It is true that chorionic gonadotropin will repair the ovarian interstitial tissue in hypophysectomized rats and produce synergism and luteinization when given in combination with FSH at proper dose levels. On the other hand, it fails to act as an antagonist when given intraperitoneally in combination with FSH or pregnant mare serum. Excretion experiments in normal rats indicate that chorionic gonadotropin passes through the kidney easily while injected pituitary ICSH cannot be recovered from the urine.[6] We (5) have also been unable to demonstrate antagonism between pregnant mare serum and gamone (the gonadotropic material excreted during and after menopause and containing both FSH and ICSH) and prospermine (from male urine, containing mainly ICSH and perhaps traces of FSH).[7]

The observations recorded agree with the conception that the primary sex development is controlled by two AP factors. We feel that the results of our quantitative study of their combined physiological action should help to clairfy some of the controversies in this field of research.

Relationship of the anterior pituitary to the adrenals and thyroids. It is generally recognized that hypophysectomy will produce atrophy of the adrenal cortex and that adrenalectomy will cause changes in the histological picture of the anterior pituitary (3). Injection of certain AP preparations containing the adrenotropic factor have been found to prevent the atrophy resulting from hypophysectomy or to cause hypertrophy of the cortex in normal animals. Adrenotropic fractions of high potency have not been reported so far and no satisfactory physiological test for adrenotropic activity has as yet been devised. The weight increase of the adrenals in normal animals cannot be regarded as specific. The histological changes and weight increase produced in the adrenals of the hypophysectomized rat may be considered at present as the most accurate test for adrenotropic fractions. In view of the biological importance of this principle it seems highly desirable to work out a more sensitive test method and to attempt further chemical purification of this factor (see addendum).

Harrop and his associates (10) have shown that the secretion of the adrenal cortex exerts an influence on the electrolyte and water balance in the body. According to recent findings by various investigators this gland also plays a very important rôle in carbohydrate and probably also in fat and protein metabolism (1, 2, 11, 15, 30).

In regard to the thyroid gland a similar interrelationship to the anterior pituitary can be observed. The secretion of the thyroid (thyroglobulin, thy-

[5] The term 'chorionic gonadotrophin' has been proposed recently by the Permanent Commission on Biological Standardization of the Health Organization of the League of Nations.

[6] Unpublished work carried out in collaboration with Dr. H. M. Evans and Dr. M. E. Simpson at Berkeley ,California.

[7] The term ICSH is used only to indicate the ability of the substance to repair the deficient interstitial tissue in the hypophysectomized rat and does not imply that these principles are identical.

roxine) is known to increase the metabolic rate and to have an influence on carbohydrate, fat and protein metabolism. Investigations which we have carried out during recent months in collaboration with H. J. F. Grattan cast doubt on the assumption that the thyroid stimulating effect of the pituitary is produced by a separate principle acting on the thyroid gland only. We have observed that the thyrotropic effect of the pituitary is always associated with the interstitial cell stimulating principle. We have prepared thyrotropic fractions by various chemical methods and tested their effects on the thyroid weight and histology of day-old chicks according to Smelser (12) and in addition on the ovarian interstitial tissue and the thyroid histology of hypophysectomized rats. The results of these comparative physiological studies lead us to believe that the two factors are identical, the effects depending on the dose level and route of administration.

Prolactin (lactogenic principle).[8] Lactation is a complex physiological phenomenon which as we now know is governed by a number of factors (1, 15, 30). The development of the mammary gland takes place in two steps which are correlated with the follicular and corpus luteum phases of the ovary. The presence of the anterior pituitary is essential to initiate and maintain lactation following pregnancy. This is indicated by the observation that hypophysectomy of lactating animals will stop milk secretion and that hypophysectomy of pregnant animals will prevent milk secretion. An AP fraction has been obtained which will cause lactation in normal female and male animals. In hypophysectomized animals, however, lactation will take place only if prolactin is supplemented either by the adrenal cortical or the adrenotropic hormone.

A sensitive test for prolactin has been found in its capacity to promote growth of the pigeon crop gland upon local intradermal administration (13). Crystallization of this pituitary principle was reported last year by White, Long and Catchpole (14); this work, however, has not yet been confirmed by other investigators.

Influence of the anterior pituitary on growth. The biochemical aspect of growth has been approached from different points of attack, namely by studying *a*) the rôle of certain amino-acids in the diet (16), *b*) the importance of vitamins in the diet and *c*) the influence of the endocrines.

Clinical observations on the disturbances of growth in man have, in many cases, been traced to a dysfunction of the pituitary. These findings can be correlated with similar observations obtained in animal experiments. Stasis of growth is observed in immature animals after hypophysectomy which can be overcome by daily implants of pituitary glands or by injection of certain AP preparations. Giant rats can be produced by prolonged injection of an alkaline pituitary extract. Whether the anterior pituitary secretes a specific separate growth principle is still unsettled at the present time; the mechanism of the hypophyseal growth effect is as yet little understood. It is quite possible that further research will show that this influence on growth is due to the combined synergistic response of those pituitary

[8] In view of the fact that this pituitary principle is only one of several factors involved in the phenomenon of lactation, the term lactogenic hormone seems to be misleading.

principles which stimulate the various other endocrine glands. It may be mentioned, in this connection, that inhibition of growth can be observed in cases of underfunction of either the thyroids or adrenals. The influence of the thyroid gland is emphasized by the recent findings of Salmon (17) that the retardation of growth resulting from thyro-parathyroidectomy of new-born rats can be remedied by administration of thyroid substance and parathyroid injection while pituitary implants or extracts have no effect if the animals are completely thyroidectomized.

Relationship of the Anterior Pituitary to Metabolism

Knowledge of the complex relationship of the anterior pituitary to carbohydrate, protein and fat metabolism is at present still incomplete. It is as yet not known whether any of the AP principles exert a direct effect on certain phases of the metabolism of foodstuffs or whether this influence is solely mediated through certain other endocrine organs, like the adrenals, thyroids and perhaps also the gonads.

That the anterior pituitary is associated in some way with metabolism, especially that of carbohydrate, is shown by the following observations.

(*a*) It has been found that a diabetogenic-like effect can be produced in normal dogs by the injection of increasing amounts of an alkaline AP extract over a period of several days. It has also been recorded that the diabetic condition persisted in some dogs after injections had been discontinued, (18, 19, 20). The assumption that the cause of the permanently diabetic condition, sometimes observed, is due to a decreased functional activity of the islands of Langerhans is supported by histological evidence, indicating exhaustion and degeneration of the islet tissue. These observations fall in line with earlier findings of Allen on partially depancreatized animals kept on a high carbohydrate diet (21).

(*b*) Hypophysectomized animals are more sensitive to insulin, indicating that the secretion of the anterior pituitary exerts an anti-insulin (glycotropic) effect (1, 11, 15, 30).

(*c*) The symptoms of experimental diabetes (removal of the pancreas) are ameliorated following hypophysectomy. The diabetic symptoms are manifested again on injection of AP extracts into such doubly operated animals. Removal of the adrenals has also been found to alleviate the symptoms of experimental diabetes. On administration of active preparations of the adrenal cortex to adrenalectomized—depancreatized animals a reappearance of glycosuria can be observed (22). Houssay and his associates have been able to demonstrate the diabetogenic action of AP extracts also in adrenalectomized-depancreatized dogs (23). In order to explain the diabetogenic properties of AP extracts, two possibilities have to be considered, *a*) that this effect is mediated solely through the other endocrine glands and *b*) that the pituitary, in addition, may exert a direct effect on metabolism (liver). For further details on this subject the reader is referred to various monographs (1, 2, 11, 15, 30).

Brief reference may be made to the following physiological effects ascribed to the anterior pituitary. Hypophysectomized animals show a greater loss

of their glycogen stores during fasting than normal animals. Russell has reported that loss of muscle glycogen, but not of liver glycogen, can be prevented by giving AP extracts to hypophysectomized animals and postulated a 'glycostatic' principle of the anterior pituitary (24). It appears quite possible, however, that this pituitary effect is produced by the adrenotropic principle inasmuch as Long and Katzin have observed that injection of cortin into hypophysectomized rats will preserve the glycogen level in the muscle as well as in the liver during fasting (25). The findings of Bennett, however, do not seem to support this assumption (26).

Young has reported that injection of certain anterior pituitary preparations renders the normal animal more resistant to insulin and proposed the term 'glycotropic' for this effect (27). The correlation of the glycostatic, glycotropic and diabetogenic effects of the anterior pituitary has not yet been worked out on a quantitative biological basis.

It has been demonstrated that the injection of certain AP extracts into normal fasting animals is followed by an increased urinary excretion of acetone bodies (1, 2, 11, 30). It seems unjustified, however, to postulate from these observations the existence of a separate ketogenic principle in the anterior pituitary since changes in fat metabolism may very well occur as a sequel to abnormalities of carbohydrate metabolism (28).

A discussion of various other physiological actions attributed to the anterior pituitary such as changes in the amounts of liver fat and blood lipoids or the pancreatropic and parathyrotropic effects has been omitted. It may be mentioned that Collip and his associates recently reported on a specific metabolic principle present in the pars intermedia of the pituitary gland (29).

The assumption that the multiple effects of AP extracts upon metabolism are due to separate principles does not seem justified at present since as yet no definite proof of their separate existence has been secured. In the hope of contributing to the clarification of this important problem, we are at present engaged in the further chemical fractionation of different AP factors and are studying their physiological response from various aspects.

Conclusions and General Remarks

It is evident that the functional integrity of the various endocrine organs is dependent on the anterior pituitary. In regard to the effect of the pituitary on metabolism it is still unsettled whether this influence is solely mediated through the various other endocrine organs or whether the pituitary gland also exerts a direct effect on metabolism (liver). It is generally recognized that metabolism is controlled not by a single factor but by the physiological coordination of various active agents in the body. In this connection one must also consider the possibility of synergistic effects as well as antagonistic actions between certain AP factors and between these AP principles and the secretions of other endocrine glands. Different physiological effects observed may also be due to the same principle, depending on the mode of injection and the dose level.

We feel that so far evidence for the existence of only the following AP principles has been obtained: *a*) follicle stimulating hormone, *b*) interstitial

cell stimulating hormone which appears to be identical with the luteinizing hormone, antagonist and thyrotropic hormone, *c*) prolactin and *d*) the adrenotropic principle. The definite proof that only a comparatively small number of active separate principles are secreted by the anterior pituitary would fall in line with cytological studies of this endocrine organ. The presence of three different types of cells has been recognized in this gland *a*) chromophobes, *b*) eosinophiles, *c*) basophiles, only the last two have been reported to show secretory activity (3).

As yet, with the possible exception of prolactin, no anterior pituitary hormone has been obtained in a sufficiently pure form to allow its chemical identification. The evidence at present indicates that the AP principles are of protein-like nature or else are closely related to proteins. The indication that the anterior pituitary secretes only a comparatively small number of separate active principles should act as an incentive for further attempts of their chemical fractionation and purification and should also greatly help in the final solution of the problem of pituitary physiology.

ADDENDUM

In collaboration with J. F. Grattan we have studied recently the glycotropic (anti-insulin) effect of various hormone preparations in mice, using the procedure suggested by J. Campbell (*Endocrinology* 23: 692. 1938). We have found that adrenotropic A P fractions as well as adrenal cortical extracts and crystalline corticosterone acetate (kindly supplied by Dr. E. C. Kendall) produce a definite glycotropic (anti-insulin) effect. On the other hand, administration of purified thyrotropic (ICSH) and prolactin preparations and also thyroxine fails to render the animals resistent to insulin under the experimental conditions employed. These results suggest that the glycotropic effect of the anterior pituitary is produced by the adrenotropic factor. We are now studying the possibility of using the anti-insulin effect as a test object for the physiological activity of adrenotropic and adrenal cortical preparations.

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CRYSTALLINE INSULIN

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SINCE THE FIRST clear demonstration by Banting and Best in 1921 (1) that there can be prepared from pancreas an extract which effects carbohydrate metabolism, much interest has centered around the chemistry and the physiological properties of the active principle. Although study of its function and mode of action provide fascinating problems, I shall confine my remarks in the present symposium to the chemistry of this hormone and shall divide my subject matter into two main subdivisions, (*a*) insulin crystals and methods used to isolate them and (*b*) the chemistry of crystalline preparations of insulin.

Insulin was first isolated in a crystalline form by Abel in 1926 (2, 3). This was the first instance of a hormone having the properties of a protein being prepared in a crystalline form. For this reason great credit must be given to the late Professor Abel and also to those who were associated with him for their brilliant work not only in isolating the crystals but also for their numerous contributions on the chemistry of this hormone. Abel's method consisted essentially of isoelectric precipitation of insulin from highly buffered aqueous solutions containing ammonium acetate, brucine and pyridine. The crystals which separated were recrystallized either from a similar buffered solution containing no brucine, or from a sodium phosphate solution. This method is somewhat tedious and time consuming, particularly if large quantities of crystals are desired. Further, the yields of crystals are often very poor. Harington and I, in 1928, succeeded in isolating insulin crystals by another method (4). We used a buffered solution of ammonium acetate or sodium phosphate to which was added insulin and saponin. We attributed the success of the method to (*a*) the power of saponins to inhibit precipitation and thus promote supersaturation and (*b*) the ability of saponins to effect a sharpening of the isoelectric points of the mixture of proteins with the result that it became possible to fractionally precipitate the proteins. Again this method proved unsatisfactory in that different samples of saponin behaved quite differently in their power to promote crystallization and, furthermore, the yields of insulin crystals only accounted for from 5 to 15% of the crude insulin powder. A carbon, hydrogen, nitrogen and sulphur analysis of these crystals gave the following results.

TABLE 1. CHEMICAL ANALYSIS OF INSULIN CRYSTALS

C	H	N	S
49.61	6.81	14.49	3.11
49.11	6.95	14.03	—

These results are in good agreement with those previously recorded by Abel for his air-dried crystals. Further, the crystals had the same microscopic appearance as those previously isolated by Abel. We also showed that different batches of insulin crystals, prepared by different methods, have the same physiological activity, namely, about 24 units per mg. During 1929 and 1930 I carried out many experiments in an attempt to improve on the existing methods of crystallizing insulin. It was found that with certain preparations of insulin, good crystals could be obtained from a phosphate buffer solution containing 10% acetone. Further experimental work indicated, however, that the ability to obtain crystals of insulin depended upon the presence of some substance apparently unknown to all of us. Since it proved impossible to attribute successful crystallization to any particular constituent of the phosphate buffer, the water or glass and since it seemed probable that inorganic substances played a definite rôle in the crystallization of insulin, there remained the possibility that the insulin or even the pancreas from which the insulin was extracted might contain inorganic substances which were, in part, responsible for successful crystallization of insulin. In searching the literature it was found that pancreas contains appreciable quantities of zinc (5) and of cobalt and nickel (6). Experiments were soon undertaken to see if these metals aided in the crystallization of insulin and it was shown that the addition of zinc to an insulin preparation from which it had previously been impossible to prepare crystals, now made it possible to obtain an almost completely crystalline product (7). The amount of zinc necessary to effect crystallization was about 2 mg. per 100 cc. of 20-unit insulin, that is, 1 mg. of zinc per 1000 international units. The crystals obtained by this method had the same microscopic appearance and physiological activity as those previously isolated by the pyridine-brucine method of Abel or by the saponin method reported by Harington and Scott. Further, the sulphur, nitrogen, carbon and hydrogen analysis of these crystals gave values which were in good agreement with those found with crystals obtained by the other methods. In view of the successful and apparently essential use of zinc in this experiment, it was a matter of interest to find that a sample of saponin which had been previously shown to be very effective in promoting the formation of insulin crystals was one containing zinc. It would seem that this fact explains the value of that particular sample of saponin. Moreover, the ash of two samples of crystals which had previously been obtained from Abel gave a very positive test for zinc. Further, samples of commercial preparations of insulin were examined and likewise found to contain zinc though the amount of this metal varied from one sample to another. This, of course, is a fact not unrelated to previous findings that the early methods for the preparation of crystals could be used with reasonable success with some brands of insulin though not with others.

Similar experiments were then carried out using nickel and cobalt instead of zinc. These metals in the same concentration as in the zinc experiments, likewise greatly facilitated the formation of crystals. The effect of other elements was next determined. It was found that cadmium facilitated greatly the formation of insulin crystals and that all other elements had no beneficial

effect. In spite of these experiments, however, one might say that the important governing factor was the purity of the material from which it was desired to obtain crystals. In other words, one might expect that, without the addition of metal, crystals could be readily obtained from an amorphous preparation provided the amorphous preparation contained 22 to 24 U of insulin per mg. as do crystals. Accordingly a quantity of insulin was first crystallized by means of zinc. The zinc was then removed from the crystals by acid acetone and electrodialysis. The resultant product contained only 0.04% ash. It was found that only those preparations of insulin to which either zinc, nickel, cobalt or cadmium had been added would yield crystals

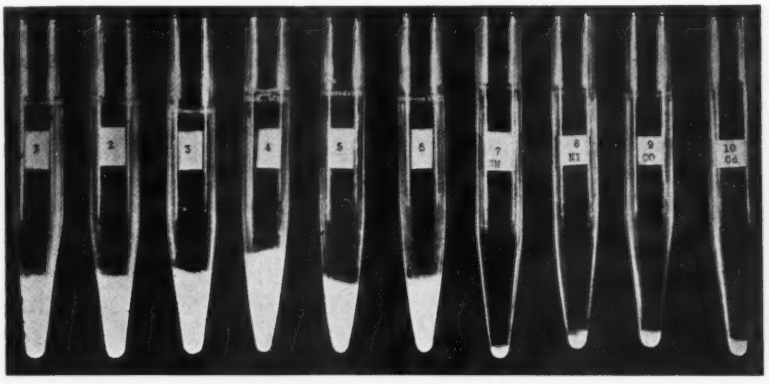

Fig. 1. Attempts to Recrystallize Insulin of Low Ash Content. Tubes 1–6 contain insulin precipitated from phosphate buffer solutions adjusted to various acidities. All precipitates were amorphous.

In tubes 7–10 are precipitates prepared from buffered solutions of insulin containing traces of the metals as indicated. The solutions were adjusted to the same acidity as the solution in tube 6. These precipitates were crystalline.

(fig. 1). Thus these experiments showed that there were at least four metals, namely zinc, cadmium, nickel and cobalt which aided in the preparation of crystals, and three of these (zinc, cobalt and nickel) have been reported to be natural constituents of the pancreas.

This finding that insulin can be readily crystallized by the presence of traces of zinc has also a practical value. It is now economically feasible to provide solutions of zinc-insulin crystals for general clinical use and such solutions have been on the market for some time. Figure 2 shows the typical appearance of insulin crystals.

These crystals are slightly doubly refractive and have a refractive index of 1.58. Examined under a microscope, the crystals present the appearance of cubes or rhombohedra approximating to cubes which are standing on one corner, thus giving rise to a hexagonal outline when first examined. The crystals form best at pH 5.8 to 6.2. This is above the isoelectric point of insulin which was found by Wintersteiner and Abramson to be 5.3 to 5.36 (8).

When a suspension of insulin crystals is viewed under a microscope, the crystals appear as has been shown in figure 2. If, however, the liquid in which the crystals are suspended is made to flow from one side of the slide to the other, the crystals move and in so doing roll over and expose various faces to view. Arising out of the examination of these rolling crystals Dr. J. Craigie and the author prepared a moving picture showing the various faces of the crystals. Figure 3 is a series of frames taken from the film and shows various consecutive positions of an individual crystal as it rolls across the field. From

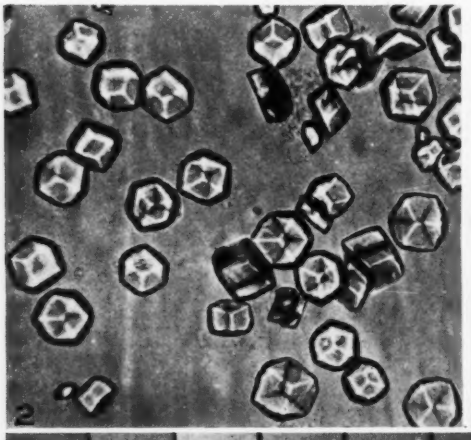

Fig. 2. Zinc-insulin crystals, formed pH 6 (approx.).
Fig. 3. Typical zinc-insulin crystal (as in Fig. 2).
Fig. 4. Zinc-insulin crystals, formed pH 5 (approx.).

this plate it will be observed that the crystal presents appearances quite unlike that commonly observed. It will be noted as the crystal rolls over that it appears as an extremely flat, plate-like rhombohedron. Crystals which normally appear to stand on a corner when viewed under a microscope are in all probability resting on one of the rhombohedral faces. Further, it will be observed from figure 3 that the insulin crystal is in reality a twin, and that twinning has occurred along the trigonal axis. In the film, from which this plate was taken, an occasional crystal is seen in which twinning has occurred at right angles to the trigonal axis. This twinned appearance of insulin crystals is the rule rather than the exception, but of course is not observed unless the crystal is moved to expose various faces to view.

In figure 3 it will be noted that the crystal first appears as a cube; as it rolls over, it exposes other faces to view, presenting the appearance of a

twinned-crystal as shown in the central frames; upon further rolling, the crystal again assumes the appearance of a cube resting upon one corner.

When attempts are made to crystallize insulin below its isoelectric point, dog-tooth crystals are obtained as shown in figure 4. Very often these crystals occur as twins and present a dumb-bell appearance (9). These crystals separate at pH 5.2. They can readily be converted into the typical rhombohedral insulin crystals by adjusting the reaction of the mixture to pH 6.2.

At this point I should like to mention briefly a second practical application that was the outcome of our work with zinc and insulin.

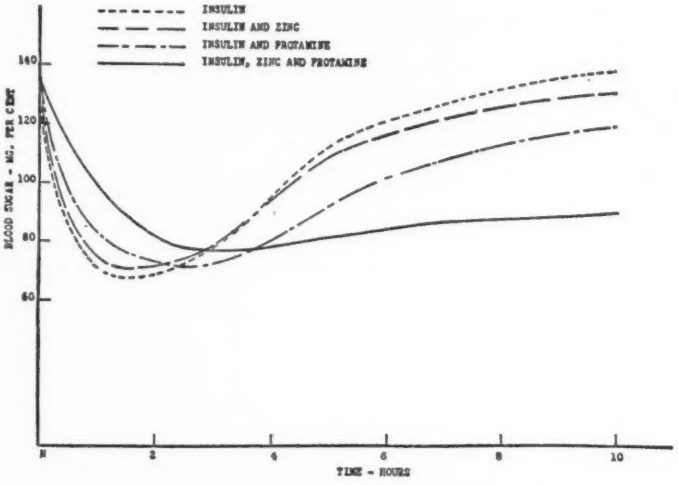

Fig. 5. Level of blood sugar in rabbits following the injection of preparations A, B, C and D.

In 1936 it was shown by Hagedorn et al. (10) that when a solution of protamine in a sodium phosphate buffer is added to an insulin solution, an insoluble complex forms at pH 7.2 which, when administered to diabetic patients, produces a prolonged hypoglycemia. From our previous work with insulin and zinc it was thought that this metal might be concerned in a union of the protamine with the insulin-protein. Our reasons for this assumption arose from a consideration of the several relationships already known to exist between zinc and insulin, namely zinc occurs in the pancreas in relatively large amounts (11) and in many commercial samples of insulin, secondly zinc readily combines with insulin as has been shown in crystallization experiments and finally it is well known that zinc forms many complex salts with basic substances such as amines which may, to some extent, be considered as resembling protamine. To test this hypothesis, samples of insulin and protamine were prepared to be of low ash content. From these materials four preparations were made (A) a solution of insulin, (B) a solution of insulin containing 1 mg. of zinc per 500 U of insulin, (C) a suspension of

insulin and protamine and (D) a suspension of insulin and protamine containing 1 mg. of zinc per 500 U. Each of these preparations was tested on 20 rabbits and the average blood sugar values are recorded (12) in the following series of graphs (fig. 5).

From these results it will be seen that the addition of a small amount of zinc to the protamine-insulin mixture greatly sustained its hypoglycaemic action, and the results suggest that zinc, or some other metal, is largely responsible for the combination that results in the prolonged hypoglycemia produced by insulin with protamine. This investigation proved to have also a very practical value since it provided a reasonably stable product and permitted the marketing of a one-vial package instead of a two-vial package (one vial containing an acidic solution of insulin and the other containing alkaline buffer solution of protamine). These solutions had to be mixed in proper proportions either by the patient or the physician before use. The protamine zinc insulin suspension was much more stable than the protamine-insulin preparation and did not show the tendency to clump or to stick to the sides of the container as was commonly observed in preparations not containing the added metal.

In regard to the chemical properties of crystalline insulin, it may be said that it exhibits all the properties of a typical protein. It is precipitated from aqueous solution by the usual protein precipitants such as sodium chloride, ammonium sulphate, tungstic acid, tannic acid and trichloracetic acid. The Pauly, biuret, Millon, ninhydrin and xanthroproteic reactions are positive. The more specific color reactions for arginine, tyrosine and cystine are positive. The tests for tryptophane and carbohydrate groups are negative. Phosphorus is not a constituent of the protein. Solutions of crystalline insulin are levorotary as are those of all proteins. Insulin is precipitated from aqueous solution by adjusting the hydrogen ion concentration to about PH 5. This was first observed by Somogyi, Doisy and Shaffer (13) and independently by Shonle and Waldo (14). This observation has been of great practical importance in the preparation of commercial insulin and in scientific researches on this hormone.

Molecular weight estimations of insulin crystals have been made by various investigators. Freudenberg and his collaborators (15), using purely chemical evidence, estimated its molecular weight as approximately 20,000. Sjögren and Svedberg (16), using the ultracentrifuge, estimated it as 35,000, similar to egg albumen and Bence-Jones protein. Gerlough and Bates (17), by viscosity measurements, estimated the molecular weight as 40,000 to 50,000.

In view of the above findings for the molecular weight of insulin, I should like to consider briefly the results of the determination of the ash content of various metal salts of insulin. In these experiments crystalline preparations of insulin were prepared from ash-free material by means of zinc, nickel, cobalt and cadmium. The various preparations were dried and the ash of each estimated (18, 19). From the results of these estimations the values shown in table 2 have been calculated.

TABLE 2. METAL CONTENT OF ZINC-INSULIN CRYSTALS, NICKEL-INSULIN CRYSTALS, CADMIUM-INSULIN CRYSTALS AND COBALTOUS-INSULIN CRYSTALS

Zn	Ni	Cd	Co
%	%	%	%
0.48	0.40	0.78	0.45
0.45	0.41	0.72	0.46
0.58	0.39	0.81	0.41
0.52	0.42		
0.44	0.41		
0.56	0.41		
Mean 0.52	0.41	0.77	0.44

If it is assumed that the formula weight of insulin is 20,000 as suggested by Freudenberg, then from our data it follows that each formula weight of zinc-insulin crystals contains 1.6 F.W. of zinc, each F.W. of cobalt-insulin crystals contains 1.5 F.W. of cobalt, each F.W. of cadmium-insulin crystals contains 1.4 F.W. of cadmium and each F.W. of nickel-insulin crystals contains 1.4 F.W. of nickel. If, however, the formula weight of insulin is about 40,000, then it is evident that the metallic content of the crystals can be expressed as formula weights by whole numbers (namely 3) within the limits of experimental error. Further, from these ash estimations it seems highly probable that insulin crystals contain zinc, cobalt, cadmium or nickel as an essential constituent and in a characteristic amount.

The results of the nitrogen distribution of hydrolyzed crystalline insulin as obtained by the Van Slyke method are shown in table 3. These results were obtained by Wintersteiner, du Vigneaud and Jensen (20).

TABLE 3. NITROGEN DISTRIBUTION IN CRYSTALLINE INSULIN

Nitrogen components	Milligrams		% of total N	
Ammonia-N	12.43		9.58	
Humin-N (On lime)	1.87			
Humin-N (H_2)	1.00	3.14	2.42	
Humin-N (H_3)	0.27			
Arginine-N	8.56		6.60	
Cystine-N	7.88	29.88	6.08	23.04
Histidine-N	9.86		7.60	
Lysine-N	3.58		2.76	
Amino-N in filtrate	73.00	83.77	56.30	64.61
Nonamino-N in filtrate	10.77		8.31	
Nitrogen recovered	129.22		99.65	
Nitrogen in 225 cc. of hydrolysate	129.70		100.00	

While the nitrogen distribution of crystalline insulin does not differ markedly from that of any other protein, yet certain values in this table might be briefly mentioned. It will be noted that the values for the ammonia nitrogen and the cystine nitrogen are higher than those obtained for most proteins.

Approximately 95% of the amino acid constituents of the molecule of crystalline insulin have been identified. The nature and the amount of the various amino acids thus far isolated are shown in table 4. These values were obtained chiefly by du Vigneaud, Jensen and Wintersteiner (21).

From this table certain values are rather suggestive as to the nature of this protein. It should be noted that crystalline insulin contains rather large amounts of cystine, glutamic acid and leucine. The results of Harington and Mead (22) would suggest even a higher value for glutamic acid, namely 30%. In regard to cystine, du Vigneaud (23) has shown that all the sulphur (3.2%) of insulin is present as a disulphide linkage. The sodium nitroprusside test for sulphydryl groups is negative but becomes positive after the reduction of the insulin molecule. If the total sulphur of insulin (3.2%) occurs as cystine then this amino acid should constitute 12% of the molecule.

TABLE 4. PERCENTAGE DISTRIBUTION OF AMINO ACIDS IN CRYSTALLINE INSULIN

Amino acids	Per cent	Method of determination
Tyrosin	12	Colorimetrically, Folin-Looney
Cystine	12	Calculated from total S content (3.2%)
Glutamic acid	21	Calculated from amide N of Van Slyke N distribution
Leucine	30	Isolated as such
Arginine	3	Calculated from Van Slyke N distribution
Histidine	4	Calculated from Van Slyke N distribution
Lysine	2	Calculated from Van Slyke N distribution
Proline	10	Calculated from nonamino nitrogen, Van Slyke N distribution
Phenylalanine	1	Isolated as phenylhydantoin
Total	95	

Earlier workers, using the Sullivan method, obtained markedly lower results. However, the more recent work of Miller and du Vigneaud (24), by modifying the method of digestion, obtained a cystine value of 12.5%. This would thus indicate that all the sulphur in insulin is present as cystine. On the other hand, Kassel and Brand (25) obtained evidence that 5% of the total sulphur in insulin is present as methionine.

In view of the fact that enzymes break down proteins into amino acids or polypeptides, a summary of certain experiments with enzymes seems desirable at this point. Insulin is hydrolyzed by pepsin, trypsin-kinase, and papain, and at the same time the physiological activity is permanently lost. The potency of the insulin is therefore related in some manner with the intact structure of the protein. During the hydrolysis of the insulin with any one of the enzymes, the physiological activity disappears much more rapidly than does the digestion of the protein. This fact would suggest that those linkages which are first attacked by the various enzymes are of especial significance for the physiological action. With this in mind an attempt was made by Fisher and Scott (26) to follow the tyrosin content of insulin during hydrolysis by pepsin. In these experiments this amino acid was one of the first to be split off, whereas the cystine content of the protein practically remained unchanged during the early stages of digestion. It could not be established, however, that the loss in activity was directly related to the tyrosin fraction.

I shall now discuss briefly a few experiments in which the reagents used are more or less specific in their action on insulin. These experiments are of particular value in that they are suggestive of certain groups or groupings

in the insulin molecule which are at least in part responsible for its physiological action. If insulin is heated in dilute alkali (N/30), it is irreversibly inactivated with the simultaneous liberation of ammonia and sulphur (27). The inactivated product shows a decreased amino nitrogen content and the cystine content is lowered to about one-half its original value. The tyrosin and arginine contents are not affected. The amount of ammonia liberated would seem to be dependent not only upon the presence of certain free amino groups but also upon the existence of certain dithio linkages in the molecule.

It was shown by Carr et al. (28) that when insulin is treated with N/10 hydrochloric acid ethyl alcohol that the insulin becomes inactivated and that activation can be accomplished by treating the resultant product with dilute alkali. Further, a part of this reactivated insulin can be crystallized (29). The crystals are of the same form and potency as the original insulin crystals. The view held by the original investigators was that esterification had taken place. However, no evidence of such has been found by analytical procedures (29) and, further, the more recent work has shown that inactivation of the insulin can also be accomplished with such solvents as acid acetone (18). Inactivation therefore cannot be due to esterification but probably involves a reversible intramolecular rearrangement of the insulin molecule.

By treating insulin in the cold with acetic anhydride an acetylated product is obtained which has a greatly diminished biological activity (30, 31). This product, however, can be partially reactivated by treatment with dilute alkali. Under the conditions of the experiments acetylation may occur at hydroxyl, amino or imino groups. Freudenberg et al. (30, 32) believe that the reactivation of the insulin by treatment with dilute alkali is associated with the hydrolysis of acetylated hydroxyl and imino groups rather than of the amino groups. This view, however, scarcely fits in with the observed decrease in amino nitrogen after acetylation and the increase in these groups after reactivation with dilute alkali. Stern and White (33) have recently shown that under certain conditions insulin can be acetylated by ketene yielding a product in which only the amino groups have been acetylated and which shows no appreciable loss in biological activity. However, when the conditions of the experiment were so modified that the hydroxyl groups of the insulin molecule were acetylated the physiological activity was found to decrease proportionately to the increase in acetylation.

When insulin is treated with formaldehyde in a faintly alkaline solution, inactivation occurs. A partial reactivation of this product can be obtained by treatment with dilute acid (32, 34). The inactivated product shows a decreased amino nitrogen and cystine content. Insulin is completely inactivated by treatment with benzaldehyde and with chlorobenzaldehyde in weakly alkaline solutions (32, 35). Jensen and de Lawder (35) ascribed this inactivation to the condensation of the aldehyde with the free amino groups of the insulin molecule. Freudenburg et al. (32, 36), however, suggested that inactivation is primarily caused by the peroxides present in these aromatic aldehydes. This latter view would explain the observed marked decrease in the cystine content.

In view of the fact that insulin contains a higher percentage of cystine than most proteins a review of certain experiments on the action of sulphydryl compounds on insulin seems of special interest. Insulin is irreversibly inactivated in faintly alkaline solutions by such compounds as cysteine, glutathione, thioglycolic acid and thiosalicylic acid. Du Vigneaud et al. (37) postulated that the effect of cysteine and glutathione on insulin was due to a reduction in the disulphide linkages. Freudenberg and Eyer (32) claim that the reduction of one or two of the total disulphide linkages in insulin is sufficient to completely inactivate insulin. Wintersteiner (38), however, found no proportionality existing between the maximal reduction and the physiological activity. Total inactivation of the insulin occurs when about one third of the total sulphur is reduced. Schock, Jensen and Hellerman (39) found that certain thioglyoxalins, e.g. thiolhistidine and ergothioneine, do not inactivate insulin in slightly alkaline solutions. Stern and White (40) have studied the course of reduction of insulin by thioglycolic acid in acid solutions. They found under the conditions of their experiment that the physiological activity of the insulin had decreased about 50% with a reduction of one or two disulphide groups of the hormone.

SUMMARY

The chemistry of insulin has been more intensively investigated than that of any other protein. Unfortunately, it has only been possible to review briefly but a few of these researches here. There are many reasons why this hormone should have received such intensive study. In the first place, the chemist is always interested in a crystalline substance. In the case of insulin, crystals of this hormone have been available since 1926 and all the evidence points towards the crystals being a chemical entity. It was the first instance of a hormone having a protein-like nature to be crystallized. Secondly, insulin has a remarkable physiological activity which can readily be measured with a fair degree of accuracy. In contrast to it one can think of certain biologically active substances which are extremely difficult to assay such as the active principle of liver extract for pernicious anaemia and the vitamins, and thirdly, as a protein it is remarkably stable. The crystals can be dried. Insulin can be precipitated from aqueous solution by various organic substances, e.g. alcohol, acetone, trichloracetic acid and also by inorganic salts without loss in potency. As a protein it is remarkably stable in acid solutions, for example solutions of insulin (PH 2.5) can be heated at 52° C. for 10 days without any very great loss in potency. It is somewhat less stable to alkali. In contrast to these properties one can think of various other crystalline physiologically active proteins which are much less stable, such as the various crystalline enzymes and the crystalline viruses.

The chemical work to date has shown that when insulin is irreversibly inactivated by enzymes or acids destruction of the physiological activity takes place at a faster rate than does hydrolysis of the protein. Further, irreversible inactivation is generally accompanied by a decrease in the cystine or in the amino nitrogen content, or in both. When insulin is reversibly in-

activated with certain reagents there is generally a blocking of either amino or phenolic groups. The reduction of certain disulphide linkages in insulin causes inactivation. These findings, however, do not always mean that other changes in the insulin molecule may not have taken place since it is not always simple, when one is dealing with a protein molecule, to correlate the action of various reagents with specific groupings. Whether the changes observed in the cystine or amino nitrogen are confined to a localized portion of the molecule which is responsible for the physiological action of insulin is unknown. Nevertheless, from the vast amount of experimental evidence it would appear that the biological action of insulin is intimately connected with the following groups:—(*a*) certain dithio groupings which are present as combined cystine, (*b*) certain amino groups which may or may not be as cystine and (*c*) certain phenolic groups which may or may not be tyrosin. When these groupings which, of course, are not specific for insulin but are found in most proteins, are affected by various chemical reagents, then reversible or irreversible inactivation occurs.

All the chemical and physical evidence points to the fact that crystalline insulin exhibits the properties of a protein. This being so, the question naturally arises as to whether the protein contains a prosthetic group which is responsible for the physiological properties of the hormone. In the case of iodothyroglobulin, the principle of the thyroid gland, the biological activity is confined to one component of the protein, namely thyroxine, which can be separated from the protein and will function alone. However, all attempts thus far to isolate a localized part of the insulin protein which possesses physiological activity have failed. On the other hand, crystalline insulin might be analogous to hemoglobin. With this protein the prosthetic group, hematin, is attached to the protein carrier and is of itself incapable of replacing the original conjugated molecule in its physiological action. Again chemical investigation has found no proof for such an analogy in the case of insulin. Accordingly, we must conclude at least for the present that the remarkable physiological activity of the hormone is due either to configuration of all the amino acids in the protein molecule or to certain specific groupings or linkages of the amino acids within the molecule. The numerous difficulties which must be overcome to determine the exact groupings are obvious to every worker in this field of biochemistry. Nevertheless, such investigations would be of great value in elucidating the chemistry of insulin and might also prove of great importance in understanding the physiological action of numerous other biologically active crystalline proteins.

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SOME ASPECTS OF THE BIOLOGY OF THE PANCREATIC HORMONES

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THE PANCREAS is a compound tubular gland arising as an outgrowth from the alimentary tract. In nearly all the vertebrates it is made up of two parts, an acinar portion and one containing the islands of Langerhans. Although the rôle of the pancreas as an aid to digestive processes was obvious at a very early date, knowledge as to its rôle as an organ of internal secretion is of relatively recent origin, and mainly the result of intensive search for the cause of diabetes mellitus.

In 1788 Cawley (1) first recorded a case in which diabetes mellitus was definitely associated with a morphological change in the pancreas; in 1877 Lancereaux (2) propounded the theory that pancreatic disease was the cause of this clinical syndrome, and in 1890 Von Mering and Minkowski (3) experimentally established the validity of this theory by demonstrating that the removal of the pancreas from the dog results in a rise in the blood sugar, glycosuria, ketonuria and an increase in the excretion of nitrogen. Three years later Lageusse (4) suggested that this metabolic disturbance was associated primarily with the islands of Langerhans, thus marking the beginning of the idea that diabetes mellitus was a disease involving an internal secretion. The isolation of insulin by Banting and Best in 1922 (5), and the subsequent demonstration by Macleod (6) that this hormone is elaborated only by the islet tissue, definitely established the place of the pancreas in the endocrine galaxy. Today it is generally believed that insulin is produced by the beta cells of the islands of Langerhans.

In more recent years studies have appeared which suggest that insulin may be only one of two or three hormones produced by the pancreas. In December 1938, Bensley and Woerner (7) reported on their studies with aqueous extracts from alpha cells of the islands of Langerhans from guinea pig pancreas. They observed that the prolonged injection of such an extract into normal guinea pigs results in an infiltration of fat into the liver, and that this infiltration could be inhibited by the simultaneous administration of glucose. The physiological significance of such a factor from the alpha cells is difficult to evaluate because of the fact that an infiltration of fat into the liver can and does occur in the absence of the pancreas. Nevertheless, Bensley and Woerner believe that the alpha cell extract may play a rôle in fat metabolism, and that it may be related to the 'fat-metabolizing' factor of Dragstedt.

More in accord with other experimental data are the studies of Dragstedt and his colleagues (8, 9), who believe that the pancreas produces a hormone which is responsible for the removal of fat from the liver. From their point of view, the fatty livers which are usually observed in depancreatized animals, even though they be maintained with insulin, are due to a deficiency of this fat metabolizing hormone which they call 'lipocaic.' However, substances such as choline, betaine and some proteins are very effective in preventing the infiltration of fat into the livers of depancreatized and normal animals and the question arises as to whether or not these substances are present in the pancreatic extract with which Dragstedt and his colleagues have been working. This is still a subject of some controversy since Best (10) claims that the activity of Dragstedt's lipocaic is due to its choline content and not to a specific pancreatic hormone.

In the absence of the pancreas, nearly all vertebrates exhibit a disturbance in the metabolism of carbohydrate, fat and protein. However, it is noteworthy that the degree of the disturbance that follows removal of the pancreas varies from species to species. Thus the cat develops a very severe diabetes associated with a marked ketosis and a tremendous glycosuria; the dog presents a much less severe picture, especially with reference to ketosis; the pig and monkey somewhat milder, the goat still more moderate, while several species of fowl may show no disturbance or only very temporary changes consequent to insulin deprivation. In view of the established observation that removal of the anterior pituitary from the depancreatized animal results in an amelioration of the diabetes, Lukens (11) suggests the possibility that the mildness of the diabetic syndrome observed in the pig and goat may be due to a low degree of hypophyseal activity in these species. That there is some interrelationship between the pancreas and the hypophysis is now generally acknowledged but the nature of this relationship is still an open problem.

In attempting to evaluate this interrelationship, we must first consider some aspects of the metabolic disturbance consequent to the withdrawal of insulin. There are only two possibilities in explanation of the high blood sugar observed in the depancreatized animal. The first is a decrease in the rate of sugar utilization by the muscles and hence its accumulation in the blood (i.e., that insulin is essential for sugar oxidation), while the second is an increased secretion of sugar into the blood stream while the utilization by the muscles remains unimpaired. It is now very well established that the liver is the only organ in the body capable of secreting glucose into the blood stream; therefore, if the factor responsible for the hyperglycemia is an inability of the animal to burn sugar, then removal of its liver should not result in a rapid drop in blood sugar because the tissues are not able to burn sugar. On the other hand, if the hyperglycemia is due to an increased secretion of sugar, then removal of the liver should result in a very abrupt drop in the blood sugar since the tissues will oxidize that which is present in the blood, while no more will be coming in. That the latter is the case, was definitely established in 1913 by Macleod (12) and again in 1923 by Mann and Magath

(13), who demonstrated that the removal of the liver from the depancreatized dog results in the development of hypoglycemia to the same rate and degree as in the normal dog. Hence, it is probable that the hyperglycemia of the depancreatized dog is due to an excessive production of sugar by the liver and not to an impairment in oxidation by the muscles.

In view of the fact that the stores of carbohydrate are inadequate to account for the large quantities of glucose secreted by the liver into the blood of the depancreatized dog, it is obvious that the extra glucose must take origin from protein and fat. That the amino acids from endogenous protein can be a source of glucose is generally acknowledged, but it is still controversial as to whether the same is true for the fatty acids. Much indirect evidence exists in support of the hypothesis that gluconeogenesis from fatty acids does occur in diabetes mellitus, but no direct evidence was available until a year ago when Blixenkrone-Moeller (14) demonstrated that glycogen can be formed from butyric acid in the perfused cat liver. Accordingly, irrespective of the mechanism whereby insulin deprivation induces an excessive production of glucose, it is obvious that the presence of precursors of carbohydrate is essential.

When the pituitary is removed from the depancreatized dog, the sugar and acetone body excretion drops and the animal can be maintained without insulin. If such an animal be deprived of food for only a few days, hypoglycemic shock may ensue. If, on the other hand, the animal is given a high protein or carbohydrate diet, it will excrete large quantities of glucose in the urine. The feeding of fat will not prevent the development of hypoglycemia, nor will it induce a ketonuria (15).

In view of these findings, the effect of hypophysectomy can be attributed to disturbances either in the mechanism responsible for the intraconversion of foodstuffs, or in the rate of mobilization of such foodstuffs from the depots to the liver. That the latter is more probable is indicated by a number of observations. Barrett, Best and Ridout (16) demonstrated that when an extract of the anterior pituitary gland is administered to rats whose fat depots are made up of fatty acids containing deuterium, the labeled fat can be found in the liver. This means that the pituitary extract stimulated the mobilization of fat from the depots to the liver. From such an observation it is permissible to assume that when the pituitary is removed from the depancreatized dog, the rate of mobilization of fat is decreased and therefore fatty acids are not available in adequate amounts for conversion to sugar or for acetone body production.

That this also may be true for endogenous protein metabolism is suggested by a number of other facts. When the hypophysectomized dog is fasted, its nitrogen excretion is extremely low while hypoglycemia develops. However, when such an animal is fed lean meat, which you will recall is absorbed as amino acids, the excretion of nitrogen rises and hypoglycemia does not develop. In the hypophysectomized-depancreatized dog, the level of the blood sugar can be raised or lowered by administering larger or smaller amounts of protein (15). Therefore, it is suggested that the animal can utilize

amino acids for gluconeogenesis, but probably cannot effectively mobilize them from endogenous stores. In another series of experiments which we recently performed, we were led to similar conclusions.

The administration of an extract of the anterior pituitary gland to a normal animal results in a retention of nitrogen in the tissues for protein synthesis as was demonstrated by Gaebler (17), Lee (18) and others. In a study of the mechanism whereby nitrogen sparing occurs in consequence of anterior pituitary administration, we observed that whereas such an extract will cause a decrease of protein catabolism in the intact normal animal, an increase in protein breakdown is observed as the direct effect of such an extract on the muscles. Thus, working with the eviscerated dog, which essentially is a muscle preparation, we noted that the administration of an anterior pituitary extract produces a marked acceleration in the rate of protein breakdown, whereas the reverse is observed with the intact animal. In attempting to find which organ is essential for counteracting this direct effect on the breakdown of muscle protein, it was demonstrated that in the absence of the pancreas alone, the extract produced an increase in protein catabolism similar to that observed in eviscerated animals (19).

These and other studies suggest that in the absence of the pancreas, an extract of the anterior pituitary gland will accelerate the hydrolysis of muscle protein and thus increase the mobilization of amino acids from the muscles to the liver, and make them available for utilization by the liver. They support the hypothesis that the pituitary gland plays a rôle in the mobilization of foodstuffs from depots, which, in the absence of the pancreas, can be converted in the liver to glucose and other oxidative products. If that be the case, why do anterior pituitary extracts induce protein deposition in normal animals?

Before attempting to answer this question, it may be of interest at this point to draw attention to the fact that insulin exerts an important influence in the metabolism of muscle protein. For many years it has been the general opinion among physiologists and biochemists that insulin is nitrogen sparing only in the diabetic organism; that in the normal it may actually increase the rate of nitrogen breakdown or be entirely ineffective. That this is not the real state of affairs was first suggested by Janney and Shapiro's observation (20) that the nitrogen sparing action of glucose can be enhanced by the simultaneous administration of insulin. More recently we demonstrated that when the influence of insulin on protein metabolism is studied by a method which is not dependent upon the measurement of the nitrogen excretion, a protein sparing effect is always obtained, even in the nondiabetic animals. This nitrogen sparing effect of insulin is attributed not only to a decrease in the deaminization of amino acids in the liver as was suggested by Bach and Holmes (21), but also to a definite decrease in the rate of mobilization of amino acids from the muscles (22). The fact that insulin can induce the removal of exogenous amino acids from the blood by the muscles of the eviscerated animal, suggests the possibility that it stimulates the synthesis rather than inhibiting the hydrolysis of muscle protein (23).

Now let us go back to the question concerning the fact that although the pancreas and the pituitary are antagonistic with regard to protein synthesis, nevertheless, the administration of the extracts of the anterior pituitary induces growth and the synthesis of protein in the normal organism. It would be impossible to answer this question if it were not for the probability that in the intact organism the pituitary not only stimulates the hydrolysis of protein, but also stimulates the pancreas to secrete insulin, which in turn exerts a protein sparing action. The validity of such an hypothesis is supported by the observations of Anselmino and Hoffman (24) and of Richardson and Young (25), that the administration of extracts of the anterior pituitary gland may exert pancreatropic effects.

Within the past three years, Young (26) established the fact that if a dog is injected daily with massive doses of a saline extract of the anterior pituitary gland, the animal may develop a persistent diabetic syndrome within a few weeks. The fact that such dogs reveal hydropic degeneration of the pancreas suggests that the diabetes may be due to the pancreatic changes and not to the pituitary injections per se (2–7). It is not unlikely that this hydropic degeneration may be due to excessive stimulation of the pancreas with a consequent exhaustion, just as was observed by Allen to occur in partially depancreatized dogs receiving large amounts of glucose. In favor of such a view is Campbell and Best's observation (27) that the pancreas from such a pituitary treated dog contains only minute amounts of insulin. This implies, as does our previously mentioned data, that a specific 'pituitary' diabetes probably does not occur in the presence of a normal pancreas.

According to the hypothesis which we have outlined, the administration of anterior pituitary extracts will result in a retention of nitrogen because not only does the extra insulin compensate for the direct effect of the pituitary on the muscles, but as a result of the action of insulin on the liver there also is a decrease in oxidative deamination with the resultant retention of nitrogen for synthesis of protein or storage as amino acids. If the pancreas is removed alone, the inhibition of the mobilizing effect of the pituitary on muscle protein is removed, and an increased hydrolysis will ensue, the consequence of which will be an increase in the amino acids available to the liver for conversion to glucose. If the pituitary is then removed, a decrease in protein hydrolysis will occur and thus decrease the amount of precursors available to the liver for glucose formation. In this connection it is of interest to note that Soskin and Levine (28) have demonstrated recently that the administration of thyroxine to the hypophysectomized animal will prevent the starvation hypoglycemia that usually occurs very readily. Such an observation is in accord with the view which we have discussed, for it indicates that any substances which will increase protein catabolism, as thyroxine is known to do, will make available endogenous precursors for gluconeogenesis, just as in the case of feeding meat to hypophysectomized dogs.

Thus far we have attempted to point to the suggestion that with the removal of insulin from the organism, there is an increased mobilization of foodstuffs to the liver which in turn utilizes them for the formation of glucose,

ketones, urea, etc. The question now arises as to the mechanism responsible for this activity of the liver.

It is possible that insulin plays a definite and specific rôle in the gluconeogenetic process so that in its absence an uncontrolled formation of glucose from fat and protein occurs. On the other hand, it is also possible that gluconeogenesis is secondary to some other change brought about by insulin deficiency, such as an excessive hepatic glycogenolysis.

There is much evidence favoring the hypothesis that insulin inhibits hepatic glycogenolysis rather than being responsible for glycogen formation. In support of this may be mentioned the fact that the liver of the depancreatized dog can make glycogen when treated with glucose, but that it cannot retain this glycogen unless insulin is administered. Likewise, the perfused liver from an animal which has been treated with insulin will not lose its glycogen as rapidly as will the liver from a non-insulinized animal. Furthermore, Lehmann (29) recently demonstrated that cell-free muscle extract will form hexosemonophosphate from glycogen and inorganic phosphate and that the rate of this reaction is reduced by the addition of minute amounts of insulin, which can be thus regarded as an inhibitor of glycogen breakdown in muscle. Finally, in a very recent report, Seckel (30) demonstrated that insulin will inhibit the rat liver glycogenolysis which normally occurs in surviving tissue slices suspended in a buffered salt solution. From his data, Seckel concludes that the essential action of insulin on the liver is an inhibition of glycogenolysis.

From the preceding, we believe it to be probable that in the absence of insulin, excessive hepatic glycogenolysis ensues, glycogen synthesis being relatively undisturbed. This results in a rapid depletion of liver glycogen. In view of the probability that glycogen is an obligatory step in the oxidation of carbohydrate, the removal of insulin then will result in a decrease in the carbohydrate available for oxidation by the liver, and, therefore, the liver must use other foodstuffs as a source of energy. Oxidative deamination will accelerate as will also the oxidation of fatty acids, and there will occur an increased formation of glucose, urea N and acetone bodies.

In order to put this hypothesis to test we studied the influence of glucose administration to depancreatized dogs receiving no insulin. Our studies revealed that a sparing of protein can be produced in the absence of insulin if glucose is administered intravenously in amounts adequate for glycogen deposition (31). Likewise, we also demonstrated that such treatment resulted in a disappearance of acetone bodies from the blood and urine (32). That this is due to a suppression of acetone body formation is supported by the evidence that the utilization of these substances by the muscles, is not influenced by glucose administration (33).

It is of interest to point out that although a complete cessation of ketone body formation can be produced by the intravenous administration of large amounts of glucose, the nitrogen sparing action of insulin cannot be reproduced quantitatively by glucose alone. This is in accord with the earlier considerations which led us to conclude that in the absence of the pancreas,

excessive hydrolysis of protein occurs in the muscles, a phenomenon which is independent of the glycogen content of the liver, and hence it is probable that the nitrogen sparing action of glucose that we have obtained is due only to the reduction in the rate of deaminization in the liver.

Time does not permit me to discuss the relationship between insulin and other endocrine glands such as the adrenals, nor is there time to present more data to indicate that the essential disturbance consequent to insulin deprivation is one in which the tissues and especially the liver have lost their ability to retain glycogen at normal concentrations of blood sugar, and that, therefore, a compensatory acceleration in the formation of glucose from fat and protein occurs in the liver resulting in a rise in the concentration of sugar in the blood towards levels at which glycogen can be retained. If this is accomplished as by artificially raising the blood sugar and thereby depositing glycogen in the liver, or by administering insulin which permits the glycogen to be retained at a lower concentration of blood sugar, the ketosis and some of the other aspects of the syndrome disappear. If the reverse occurs, and the loss of liver glycogen is accelerated as in phlorhizin diabetes or in hyperthyroidism, the ketosis and other aspects of diabetes are aggravated.

I realize that many of the considerations that I have discussed are far from being firmly established, and are to a large degree speculative in nature, but I have chosen to present them in the attempt to build up a general picture of the rôle of the pancreas as an endocrine gland. This may be summarized by the generalization that the pancreas tends to preserve the depots of foodstuffs in the various parts of the body, as compared with the anterior pituitary gland which tends to accomplish the reverse. So long as the pancreas is functionally intact, it is improbable that the syndrome of diabetes mellitus can be produced by any agent other than one which directly involves the function of the liver and its ability to retain glycogen. Furthermore, from this point of view, the rôle of the pancreas in the general phenomena of growth becomes of paramount importance and may explain the occurrence of dwarfism amongst diabetic children.

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33. Nelson, N., I. Grayman and I. A. Mirsky: *Proc. Soc. Exper. Biol. & Med.* 39: 51. 1938.

ASSOCIATION NOTICE

PROCEEDINGS OF THE TWENTY-THIRD ANNUAL MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

THE TWENTY-THIRD ANNUAL MEETING of the Association was held in St. Louis at the Hotel Jefferson on May 12th and 13th, 1939. One hundred and thirty-eight members and one hundred and one guests registered for the meeting.

SCIENTIFIC SESSIONS AND DINNER

President David P. Barr presided at the four scientific sessions, held in the forenoons and afternoons of May 12th and 13th. The papers listed in the program published in the May, 1939, issue of The Journal were presented.

The subscription dinner was held in the Ivory Room of the Hotel Jefferson, and was attended by one hundred and forty-six members and guests.

President Barr delivered the Presidential Address in which he recounted the growth of endocrinology within the last twenty years and pointed out the increasing tendency towards specialization in this field. He also read a letter to the Association on behalf of E. R. Squibb & Sons Company making the offer of an annual award for outstanding research in endocrinology.

The Invitation Address was presented by Professor C. N. H. Long of Yale University, on the topic "Diabetes Mellitus in the Light of our Present Knowledge of Metabolism." This was illustrated with lantern slides.

COUNCIL MEETINGS

The first Council Meeting was held at the Hotel Jefferson following breakfast on May 12th. The second Council Meeting was held following luncheon at the Hotel Jefferson on May 13th. A third brief meeting was held at 5:30 P.M. on May 13th. All of the officers and members of the Council were present. The following business matters were transacted.

1. The minutes of the last previous meeting were read and approved.

2. The matter of publishing a roster of the membership of the Association was discussed. Affirmative action was taken and the matter was referred to the Publication Board.

3. The Council discussed at length the question of place and time for the next annual meeting. It was first decided that the next meeting should be held in Atlantic City, during the same week as the meetings of the Association of American Physicians and the Society for Clinical Investigation, the exact days being left to deci-

sion later after conference with the officers of these societies. A motion to reconsider this action was carried, and a compromise motion was then adopted to the effect that the next annual meeting should be held in New York City on Monday and Tuesday of the week of the meeting of the American Medical Association; and that the meeting in 1941 should be held in Atlantic City during the week in which the meetings of the Association of American Physicians and the Society for Clinical Investigation will occur. President Barr appointed Dr. Fuller Albright and Dr. E. L. Sevringhaus as a committee to confer with the officers of these two societies, with a view to avoiding conflicts in dates and interests.

4. Dr. E. P. McCullagh and Dr. H. H. Turner were named to act as election tellers for the meeting.

5. The Treasurer's report was read and accepted.

6. A number of other matters were discussed without any formal action being taken.

7. During the year the Council elected to membership in the Association the following nominees, in the manner provided by the By-Laws.

Barlow, O. W., M.D., Rensselaer, N. Y.
Bunde, Carl A., Ph.D., Oklahoma City, Okla.
Chaves Montes, Julio, M.D., Mexico City, Mexico
Cohen, George M., M.D., Newark, N. J.
Cooper, Oscar J., M.D., Philadelphia, Pa.
Cross, James B., M.D., Buffalo, N. Y.
Cullimore, Lloyd L., M.D., Provo, Utah
Driscol, Charles D., M.D., West Collingswood, N. J.
Evans, Herbert M., M.D., Berkeley, Calif.
Evans, Gerald T., M.D., New Haven, Conn.
Fox, Ruth M., M.D., New York, N. Y.
Fraps, Richard M., Ph.D., Beltsville, Md.
Gassner, F. X., D.V.M., Fort Collins, Col.
Gerber, Philip, M.D., Philadelphia, Pa.
Gilbert, Max, M.D., Bloomfield, N. J.
Herter, Walter B., M.D., Honolulu, T. H.
Kemp, Robert S., M.D., Chicago, Ill.
Ketcham, Wm. Merrit, M.D., Kansas City, Mo.
Kinsell, Laurence W., E. Stroudsburg, Pa.
Koppe, Harold F., M.D., Dayton, Ohio
Kotas, Walter S., M.D., Greybull, Wyo.
Leathem, James H., Ph.D., New York, N. Y.
Levin, Louis, Ph.D., New York, N. Y.
MacBryde, Cyril M., M.D., St. Louis, Mo.
Meloy, Margaret B., M.D., Peoria, Ill.
Miller, R. Bretney, M.D., Boston, Mass.
Nicholson, Margaret M., M.D., Washington, D. C.
Osmon Hill, W. C., M.D., Colombo, Ceylon
Reece, Ralph R., Ph.D., New Brunswick, N. J.
Rugh, Roberts, Ph.D., New York, N. Y.
Rypins, Russel F., M.D., San Francisco, Calif.
Salmon, Theodora N., Ph.D., New York, N. Y.
Saunders, Francis J., Ph.D., Chicago, Ill.
Schallig, Dorothy W., M.D., Sacramento, Calif.
Schwartz, Irving, M.D., San Francisco, Calif.
Selye, Hans, M.D., Montreal, Canada
Sieve, Benjamin F., M.D., Boston, Mass.
Simonson, Daisy G., Ph.D., Monrovia, Calif.
Spennrath, Florence G., A.B., New York, N. Y.
Suehs, M. E., M.D., Beaumont, Texas
Szarka, Alexander J., M.D., Budapest, Hungary
Zondek, Prof. Dr. Med Bernard, Jerusalem, Palestine

The E. R. Squibb & Sons Award

President Barr read to the Council a letter from Dr. George A. Harrop formally offering, on behalf of E. R. Squibb & Sons, an annual award in the sum of $1,000 to be given for outstanding work in the field of endocrinology. This is to be known as the E. R. Squibb & Sons Award and is to be granted under the auspices of the Association to a candidate selected under conditions and regulations which the Association may establish.

The offer of the Award was accepted by the Council in the following resolution.

"Whereas, E. R. Squibb & Sons have offered an annual award in the sum of one thousand dollars, to be given at the discretion of the Association for the Study of Internal Secretions as an annual award for meritorious work in the field of endocrinology.

"Be it, therefore, resolved that the generous offer be accepted with thanks by the Association for the Study of Internal Secretions, and that a committee be appointed by the President to devise arrangements for the judicious bestowal of the award annually under the name of 'The E. R. Squibb & Sons Award.'"

Considerable discussion was then had on the following matters concerning with the award: a) age limit for the recipient; b) splitting the award among two or more coworkers; c) recency of the work to be considered; d) limitation of recipients to workers in North America; e) methods of making nominations for the award.

After this discussion a motion was adopted that the President-Elect, Dr. Philip E. Smith should appoint a committee to set up the permanent mechanism for making the award. Dr. Smith appointed the following members as a Temporary Committee to advise on the details of a permanent policy for the Squibb Award: Dr. E. L. Sevringhaus, Chairman, Dr. F. A. Hartman and Dr. David P. Barr.

Further details concerning this award will be published later in the Journal.

Report of Publication Board

The Publication Board reported to the Council that the change to monthly publication of ENDOCRINOLOGY had proved thoroughly satisfactory. When the change was proposed it was estimated that about 1,300 pages of text could be issued in 1938. Actually 1,564 pages were published, with 184 original papers and more than a thousand abstracts. In 1939 it is estimated that about 1,800 pages will be published.

The pressure of material for publication continues to increase. Endocrine research is not waning, and both clinical and laboratory research men in this field are turning more and more to the Journal as the most suitable medium for the publication of their papers. The editorial policy of the Journal is definitely committed to meeting adequately the publication needs of endocrine medicine and biology. It is considered that there are two essential corollaries to such a policy; a) that the highest standards of quality for papers accepted be adhered to; b) that acceptable material be published promptly.

To meet the second of these conditions it is estimated that not less than 2,000 pages should be issued in 1940, to include about 225 papers and abstracts. It was accordingly decided by the Publication Board that beginning with 1940 the Journal should be issued in three volumes of about 700 pages each per year. Because of our currently favorable financial condition, the subscription price per year would not be increased. The Publication Board also authorized the publication of monographs, or monograph supplements to the Journal, provided satisfactory business arrangements for their distribution can be made.

Following the discussion in the Council meeting on the above proposals, the Council adopted the following resolution.

"It is the opinion of the Council that the Journal should not be enlarged (except by more efficient format, typography, etc.) unless in the judgment of the Publication Board the amount and quality of material submitted makes such enlargement extremely desirable."

The Publication Board approved making minor changes in format and paper stock for the Journal, in the interest of economy and of reducing the bulk of each volume.

The position of 'Assistant to the Editor' was created, to replace the former position of 'Editorial Assistant.' Miss Jennie Gregory was employed in the new position.

It was recommended that a series of invited papers be published on various topics in clinical endocrine therapy. Each paper, on a given topic, would consist of discussions by several experienced workers, with a view to bringing out both the advantages and limitations of the best modern types of endocrine therapy.

A full financial report for the Journal was presented to the Council. A number of other matters were discussed by the Publication Board, without definite action being taken.

GENERAL BUSINESS MEETING

May 13, 5:00 P.M.

The minutes of the last annual meeting were read and approved.

A summary of the Treasurer's Report to the Council was read and approved.

The election tellers, Dr. E. P. McCullagh and Dr. H. H. Turner reported the following officers had been elected:

President: Philip E. Smith
President-Elect: E. L. Sevringhaus
Vice-President: Max A. Goldzieher
Secretary-Treasurer: E. Kost Shelton
Council (three years): David P. Barr
John C. Burch
E. C. Kendall

Publication Board (three years):

David P. Barr
Oscar Riddle

President Barr expressed his appreciation of the help and cooperation he had received during his term of office.

It was unanimously voted that a letter of thanks be sent to Dr. Daniel L. Sexton and to the management of the Hotel Jefferson for their most efficient and pleasing arrangements for the meeting.

David P. Barr, *President*
E. Kost Shelton, *Secretary*

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NEWS ITEMS

THE NATIONAL RESEARCH COUNCIL, upon recommendation of its Committee on Research in Endocrinology, has made grants for the year 1939–1940 for the following work.

1. *University of Buffalo.* Work under the direction of *Wayne J. Atwell.* Development of a test for thyrotropic substance in human urine.

2. *Harvard Medical School (Collis P. Huntington Memorial Hospital).* Work under the direction of *J. C. Aub.* An investigation of the growth in vitro of glands of internal secretion and the effects of injecting carcinogenic substances into the various glands of internal secretion.

3. *University of Virginia.* Work under the direction of *Sidney W. Britton.* A study of the interrelationships between adrenal and hypophyseal functions and of the effects of cortico-adrenal extracts on isolated organs.

4. *University of California.* Work under the direction of *I. L. Chaikoff.* A study of the effects and interrelations of estrogens and androgens on the lipid metabolism of the bird.

5. *University of Denver.* Work under the direction of *Fred E. D'Amour.* A study of hormonal influences on the rate of development of chemically induced tumors.

6. *University of Chicago.* Work under the direction of *Lester R. Dragstedt.* A study of lipocaic in relation to fatty infiltration of the liver and experimental arteriosclerosis.

7. *University of California.* Work under the direction of *Herbert M. Evans.* Further study of hypophyseal hormones in relation to growth.

8. *University of Chicago.* Work under the direction of *T. F. Gallagher.* Further studies on the purification of the pituitary hormones.

9. *University of Chicago.* Work under the direction of *E. M. K. Geiling.* A study of the occurrence of various hormones in the hypophyses of different animals.

10. *Women's Medical College of Pennsylvania.* Work under the direction of *B. K. Harned.* A further study of the endocrinology of a strain of rats, especially susceptible to experimental diabetes.

11. *Ohio State University.* Work under the direction of *Frank A. Hartman.* An investigation of cortin and the sodium factor used separately and jointly on adrenalectomized animals and for a study of the properties of these two factors.

12. *Northwestern University Medical School.* Work under the direction of *A. C. Ivy.* Further studies of a substance found in urine which depresses gastric secretion, and for the determination of the effects of this substance in experimental cancer.

13. *Washington University.* Work under the direction of *Leo Loeb.* Further studies on the endocrinology of old age.

14. *Yale University.* Work under the direction of *C. N. H. Long.* Further studies on the mechanism of action of the cortical hormone and of the anterior pituitary control of metabolism; the rôle of the thyroid in control of metabolism; the effect of pregnancy on the diabetes of partially depancreatized rats.

15. *University of Rochester.* Work under the direction of *J. R. Murlin.* (1) A study of the influence of insulin on the growth and metastasis of the Brown-Pearce epithelioma. (2) A study of the influence of thyroxin and thyrotropic hormone in rats of various ages.

16. *University of Rochester.* Work under the direction of *E. S. Nasset.* Further studies on enterocrinin.

17. *Wayne University.* Work under the direction of *Warren O. Nelson.* A study of the control of lactation.

18. *Cornell University Medical College.* Work under the direction of *George N. Papanicolaou.* Further studies on the endocrinology of old age.

19. *Northwestern University Medical School.* Work under the direction of *S. W. Ranson.* A study of the hypothalamus and the hypophysis in relation to obesity.

20. *Long Island College of Medicine.* Work under the direction of *Samuel R. M. Reynolds.* A further study of the vascular effects of estrin.

21. *Johns Hopkins University.* Work under the direction of *Curt P. Richter.* Further studies of the influence of hormones on appetite.

22. *Carnegie Institution of Washington, Station for Experimental Evolution.* Work under the direction of *Oscar Riddle.* A study of various hormones on various animals.

23. *Harvard Medical School (Thorndike Memorial Laboratory).* Work under the direction of *William T. Salter.* Further studies of the effects of sex hormones on the resistance to experimental tumors.

24. *University of Minnesota.* Work under the direction of *Leo T. Samuels.* A study of the relation of the pituitary to lipid metabolism.

25. *Washington University.* Work under the direction of *Philip A. Shaffer* and *Carl F. Cori.* Further studies of anterior pituitary extracts.

26. *Cornell University Medical College.* Work under the direction of *Ephraim Shorr.* Further studies of the influence of hormones on carbohydrate metabolism.

27. *Johns Hopkins University.* Work under the direction of *George W. Thorn.* Further studies of the adrenal cortical hormones on the metabolism of canine and human subjects and for a study of the steroid compounds of urine.

28. *University of Missouri.* Work under the direction of *C. W. Turner.* A study of the influence of endocrine factors on lactation.

29. *New England Deaconess Hospital, Boston.* Work under the direction of *Priscilla White.* A study of endocrine factors in the pregnancy of diabetics.

CURRENT ENDOCRINE LITERATURE

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ADVANCE ABSTRACTS

BAUER, J., W. G. FISHER AND G. GUTMAN. Studies on anti-thyroid principles. *To appear in this Journal.*

Novocain or procain lowers the body temperature of a guinea pig to a considerable degree when injected subcutaneously. Glaubach and Pick have shown that this effect does not occur when thyroxin is administered to the animals prior to the injection of novocain or procain. This fact was used as a method for studying the possible antagonistic action of certain substances toward thyroxin. Such an antagonistic principle has been found in sheep serum and human serum. Di-iodotyrosin, olive oil and theelin did not exhibit such an effect. The method used in these experiments was not appropriate for the detection of thyroid hormone in excess in the blood of hyperthyroid subjects.

BERGMAN, A. J., O. B. HOUCHIN AND C. W. TURNER. Efficiency extraction and separation of certain AP hormones. *To appear in this Journal.*

The efficiency of chemical extraction, separation and purification of certain hormones of sheep and cattle anterior pituitaries is reported. It was observed that by means of an initial extraction there was a reduction of 84 to 87% of the dry matter of the acetone dried powder, yet there remained a rather high percentage of the lactogenic, thyrotropic, gonadotropic, carbohydrate and fat metabolism hormones. The lactogenic hormone was further extracted from the initial extract and found to contain only traces of thyrotropic, gonadotropic, carbohydrate and fat metabolism hormones. An aqueous fraction precipitated with flavianic acid was found to contain practically all of the thyrotropic hormone contained in the initial extract, whereas only 50 to 60% of the gonadotropic, and 30% of the carbohydrate metabolism hormone was recovered. The fat metabolism hormone was not recovered to any extent. This is taken to indicate the separate identity of this factor.

BURDICK, H. O., AND B. EMERSON. Repression and resorption of the corpora lutea of early pregnancy following injections of testosterone propionate. *To appear in this Journal.*

Daily injections into mice of 5 mg. testosterone propionate begun the day of mating caused the corpora lutea to become corpora albicans and hastened their resorption. These corpora were barely discernible after 8 days of such injections. The ovaries were decreased in size. Similar treatment of mice with 0.25 mg. for the same length of time resulted in larger ovaries but in small and degenerated corpora lutea. While their resorption was not as rapid as in the 5 mg. series, the final changes were just as definite. Temporary sterility was established with either amount. The rate of resorption of corpora lutea was presented as a possible means for determining amounts of testosterone propionate in unknowns. Photographs of whole ovaries are included to show the effects of testosterone propionate on the corpora lutea of early pregnancy.

BUTCHER, E. O., AND R. A. RICHARDS. The relation of the adrenals to the retarded hair growth in underfed albino rats. *To appear in this Journal.*

When young albino rats whose hair growth had been greatly retarded by underfeeding were adrenalectomized, their hair buds often began active proliferation and the formation of a new hair within 40 hours after the operation. Underfeeding induced the adrenal to enlarge and its cortex to release secretory inclusions. It is interpreted that these secretory substances acted in some way to retard hair growth.

When the hair finally grew in the underfed animal, new cells were added to the zona glomerulosa from the capsule, the glomerulosa became more definitely demarcated, and the granular inclusions of the cortex greatly increased. These changes indicated that there was no longer the necessity for the adrenal to retard the hair growth. Since hair growth was induced so quickly after adrenalectomy, and the adrenal hypertrophied and changed histologically in the underfed animal, it is believed that the adrenal in some way was partly responsible for the retarded hair growth in inanition.

CRABTREE, D. G., J. C. WARD AND J. F. WELCH. Sex differences in albino rats to toxic doses of powdered red squill. *To appear in this Journal.*

Previous workers have reported that the female rat was about twice as susceptible to the rat-killing principle of red squill as was the male rat. In the present work these results were confirmed and further studies were made concerning the toxic effects of red squill upon castrate male and spayed female rats. It was found that the presence or absence of the ovary had no effect upon the toxicity of red squill to the female but when the testes were removed the castrate males were approximately as susceptible as the females. It was also found that when testosterone was supplied to the castrate males their resistance to red squill increased to the level shown by the normal males. The presence of the testes or the hormone of the testes (testosterone) is the determining factor in the increased resistance to red squill shown by male as compared with female rats.

DAVIS, H. P. Anorexia nervosa: a case report. *To appear in this Journal.*

A case history of anorexia nervosa is presented with various differential diagnostic procedures. Results of vaginal aspiration specimens and endometrial biopsy showed inactivity of vaginal mucosa and endometrium. Therapy with vitamin B and with thyroid extract and pituitary gonadotropic substance was ineffective. Complete recovery with weight gain and resumption of menses followed psychotherapy and high caloric diet. The purpose of the paper is to stress the ineffectiveness of glandular therapy in this condition, which in many respects resembles an organic endocrine dysfunction.

EVANS, H. M., M. E. SIMPSON, S. TOLKSDORF AND H. JENSEN. Biological studies of the gonadotropic principles in sheep pituitary substance.

Biological studies of two purified gonadotropic pituitary fractions, FSH and ICSH (LH) in normal and hypophysectomized immature female rats show that the phenomena of *a*) synergism (activation), *b*) luteinization and *c*) antagonism can be produced by the simultaneous injection of these 2 factors under certain conditions. The synergistic effect between the 2 factors is specific and is in contrast to the non-specific augmentation observed on the addition of certain substances (casein, zinc sulfate) to gonadotropic pituitary preparations. The ICSH fraction when given intraperitoneally inhibits the follicular development produced by either FSH or pregnant mare serum. Biological comparison between prolan and the hypophyseal ICSH principle indicates that they are different substances.

FINKLER, R. S., AND W. ANTOPOL. Histological changes in the senile vagina induced by estrogenic therapy administered orally and by inunction. *To appear in this Journal.*

This presentation deals with estrogenic therapy in 37 patients presenting symptoms of senile vulvo-vaginitis frequently complicated by vaso-motor disturbances of menopause. Twenty-one patients in this group were treated with estrogenic substances by the method of inunction and 16 patients by the oral route. The effect of intensive estrogenic therapy in this series of 37 patients was followed before, during the course of, and on the completion of therapy as well as during the control periods. Topical application of estrogens in a suitable base (lanolin) especially in patients who have none or only slight general menopausal symptoms, proved to be of marked benefit in alleviating the symptoms of senile vulvo-vaginitis and produced pronounced changes in the vaginal epithelium from the senile type to the type present during active sex life. Patients presenting inflammatory changes superimposed on an atrophic vaginal mucosa responded rapidly to local treatment. Evidence that systemic absorption takes place in the course of inunction was manifested by slight to moderate improvement in the vaso-motor symptoms of the menopause and a recurrence of uterine bleeding in one patient. Oral administration of estrogens proved also to be of great value, producing similar beneficial effects; as well as growth of the vaginal epithelium as evidenced by the changes in the vaginal smear and vaginal biopsy. This form of therapy is indicated in patients presenting marked vaso-motor disturbances in addition to the symptoms of senile vulvo-vaginitis. The preparations used by the oral route were progynon D-H Tablets (estradiol tablets), emmenin (estriol glucuronide) and estrobene (diethyl-stilbestrol). Of the 21 patients treated by inunction, 6 obtained partial relief only. In these cases a change to injection therapy was made without marked improvement except in one instance. It should, however, be pointed out that there were complicating factors present such as diabetes, eczema, extensive leukoplakia and hypertrophic pathological changes of the vulva. Of the 16 patients treated with oral medication, one failed to respond. Better cooperation on the part of the patient was obtained when the treatment was given orally.

FLEISCHMANN, W. The use of colchicine in the assay of androgens. *To appear in this Journal.*

By arresting mitosis with colchicine, a clear cut histological picture of cellular stimulation can be obtained. By this method the stimulating effect of androgens upon the seminal vesicle of the castrate rat is sharply defined. A positive test (the finding of numerous mitoses) is given by 0.5 mg. of androsterone (5 I.U. androgen) and by 0.05 mg. testosterone propionate. With smaller doses only occasional mitoses are found. The method can be used for estimating the androgenic activity of tissue or urine extracts.

FRANK, R. T. A modification of the Zondek alcohol method for determining both luteinizing and follicle stimulating principles in the urine. *To appear in this Journal.*

By modifying the Zondek alcohol precipitation method for urine by means of acidification to PH 3.5, both follicle stimulating and luteinizing factors are obtained even from castrate urine. The resulting precipitate is non-toxic to rats.

FRANK, R. T., AND R. L. BERMAN. A comparison of four methods of bioassay for the gonadotropic factors. *To appear in this Journal.*

Comparing the relative merits and disadvantages of the several procedures the authors decide to continue to use the ovarian values as their method of bioassay, because it appeared to present a more comprehensive picture of the gonadotropic forces at work.

HAMBLEN, E. C., R. A. ROSS, W. K. CUYLER, M. BAPTIST AND C. ASHLEY. Studies of the metabolism of androgens in women. *To appear in this Journal.*

Urinary androgens were investigated by a modification of the colorimetric method of Oesting in a group of 27 women who had diverse levels of ovarian function. In all, 672 24-hour specimens of urine were titrated. Daily titers of the same woman varied markedly. The use of average values of many daily determinations was deemed necessary for significant data. Essentially normal androgenic titers were obtained in the following groups of women : *a*) 5 healthy women, aged 21 to 43 years, who experienced cyclic menses; *b*) 2 women, who had had operations involving hysterectomy with ovarian conservation and 5 women, who had had operations involving hysterectomy and bilateral oophorectomy; *c*) 2 young women, aged 14½ and 19 years, who had statural and sexual retardation and postponed menarches; *d*) 2 young women, aged 17 and 29 years, who had functional menometrorrhagia associated with various degrees of endometrial proliferation and differentiation. Androgenic titers tended to be high in the following groups of women: *a*) 4 women, aged 16 to 30 years, who had had episodes of amenorrhea which ranged in duration from 4 months to 5 years; *b*) 5 women, aged 42 to 74 years, whose last episodes of uterine bleeding had occurred from 9 months to 26 years prior to the period of observation. Injections of testosterone propionate, which varied as to single doses from 10 to 100 mg. and as to number from 1 to 10, resulted in definite elevations of androgenic titers. The recoveries of the androgen injected ranged from 7.6% to 71.0%. Intensive estrogenic therapy of 2 patients caused definite decreases in androgenic titers. The following generalizations seemed warranted from the data reported. (*a*) The chief source of androgens in the female is not the ovaries, but most likely the adrenals. (*b*) The complexional 'coloring' and the androgenic titers of women apparently are related. (*c*) The increased androgenic titers of some patients who have episodes of amenorrhea may be of diagnostic, prognostic and therapeutic import. (*d*) An apparent increased production of androgens during the early post-menopausal period may signify the existence of another mechanism for stabilization of pituitary function after ovarian effects have become minimal. (*e*) The androgens may play an active rôle in the production of the regressive alterations in the sexual system of ageing women.

HARNED, B. K., AND V. V. COLE. Evidence of hyperfunction of the anterior pituitary in a strain of rats. *To appear in this Journal.*

Rats of the Yale and Wistar strains grown on the same diet and under the same conditions for 3 generations have been compared. The Yale rats exhibit: (*a*) a higher fasting blood sugar; (*b*) a lower glucose tolerance; (*c*) a greater hyperglycemic response to epinephrine; (*d*) an initial resistance to insulin; (*e*) a larger volume of urine per unit of body weight; (*f*) a more rapid growth and larger size; (*g*) a greater incidence of sterility; (*h*) a higher percentage of body fat. The authors suggest that a hyperfunction of the anterior pituitary in the Yale strain offers a common denominator for the observed differences.

HUDDLESON, J. H., AND R. A. MCFARLAND. The response of chronically fatigued neurotic patients to adrenal cortex therapy. *To appear in this Journal.*

Glycerinated adrenal cortex (glycortal pills, Schieffelin & Co.) was administered by mouth to a series of 14 patients suffering from psychoneuroses marked by asthenia. The experimental period for each patient, lasting from 10 to 15 weeks, began without medication for one week, followed by 2 weeks of placebos. These weeks, and occasionally a blank-pill week later, served as controls. The increasing

dosages ranged from 15 to 48 pills daily, each pill representing 3 grains of fresh gland. The Schneider Index, B.M.R., and weight were taken weekly for all 14 patients. A battery of physiological and psychological tests was given at the beginning and end of the experimental period to 10 of the 14. Half the patients reported subjective improvement. Half gained a kg. in weight. The B.M.R. was accelerated in 8. The Schneider Index was bettered in 9 and unaffected in 5. The inventories of psychological and of physiological complaints showed improvement respectively in 7 and 6 of the 10 patients so tested. The word association test indicated fewer neurotic responses in 7, neuro-muscular coordination was improved in 5, and perseveration was lessened in 4. Following oral medication with adrenal cortex, the objective tests for improvement in asthenic psychoneurotics gave favorable results, on the average, in over half the series. We conclude that this therapy has proved valuable in a category of obstinate disability.

LEIGHTY, J. A., AND H. J. WICK. Diethylstilbestrol compared to estrone in causing estrus in spayed mice; and in conjunction with progestin in inducing sexual receptivity in spayed guinea pigs. *To appear in this Journal.*

Diethylstilbestrol when followed by progestin was found to induce sexual receptivity in spayed guinea pigs. The results obtained either by injection or percutaneous administration of the estrogen were comparable to those obtained with estrone in the doses investigated. In spayed mice it was found that 0.05γ of estrone was one mouse unit when given subcutaneously under the conditions of our experiment. It required 25 to 30 times as much orally and 2 times as much percutaneously to get the same effect. When stilbestrol was tested in the same way it required 1.25 times as much subcutaneously, 6 times as much orally and 6 to 7 times as much percutaneously to get an estrogenic effect equivalent to that of 0.05γ of estrone subcutaneously.

ROTHERMICH, N. O. A comparative study of the effects of male and female sex hormones on the human vaginal smear. *To appear in this Journal.*

The theoretical and experimental basis for the clinical use of male sex hormone in women is critically discussed. Eleven menopausal women, institutionalized for involutional psychosis, were given injections of relatively large doses of testosterone propionate over an extended period of time. A nearly identical group received approximately similar amounts of estrone. In addition 2 patients were given both hormones simultaneously in varying quantities. Vaginal smears were studied before and throughout the entire period of treatment, and the changes produced by each hormone are analyzed singularly and by contrast and comparison. The significance of these observations is discussed in the light of other work and with a view toward understanding the collective action of male sex hormone in the human female.

RICHTER, C. P., AND E. C. H. SCHMIDT, JR. Behavior and anatomical changes produced in rats by pancreatectomy. *To appear in this Journal.*

Of 28 rats pancreatectomized at an average age of 65 days, 18 died and 10 survived during the 40-day observation period. Six of the 10 surviving rats became almost totally inactive. Their average daily activity decreased 98.8%. The other 4 became only moderately inactive, showing a decrease of 71.8%. The 6 totally inactive rats tended to maintain their operative weight; they ate less food, drank more water, showed diestrous vaginal smears and had small endocrine glands. The 4 moderately inactive rats deviated only slightly from the normal in body weight, water intake and vaginal smears. At autopsy, performed 40 days after pancreatectomy, 5 of the 6 inactive rats showed no pancreatic tissue; the other 4 rats, minute fragments. Control laparotomy experiments showed that the operative manipulation did not make the rats inactive. Control experiments were also made with 6 rats kept on a limited diet of 8 gm. per day (the average daily intake of the pancreatectomized rats). These rats still showed a high degree of activity, thus suggesting that the pancreatectomized rats did not become inactive because of the reduced food intake. Atrophy of the endocrine glands, with the possible exception of the ovaries and reduced food intake were ruled out as causal factors in producing the inactivity. It was concluded, therefore, that the depancreatized rats became inactive largely because of their inability to metabolize, carbohydrate.

SELYE, H. The effect of adaptation to various damaging agents on the female sex organs in the rat. *To appear in this Journal.*

Experiments on the rat indicate that during exposure to non-specific damaging agents (such as excessive muscular exercise, qualitatively or quantitatively inadequate diets, or toxic doses of various drugs) the ovaries undergo atrophy and more or less permanent anestrus ensues. These changes cannot merely be due to loss of weight since, depending on the experimental conditions, anestrus may occur even though the body weight increases or vaginal cycles may remain normal in spite of loss of body weight. The present paper is a study of these changes in the ovaries.

SMITH, O. W., G. VAN S. SMITH AND S. SCHILLER. The estrogens of urine from women: hydrolysis, separation and extraction. *To appear in this Journal.*

A satisfactory method for hydrolyzing urines from women and for the separation and extraction of estrone, estriol and an as yet unidentified non-ketonic estrogenic substance, 'x' estrogen, is described. The experimental results which have led to the adoption of this procedure are briefly reported. We have adhered to the Cohen and Marrian method for separating estrone and estriol fractions. Bioassay, under standardized conditions, has been used throughout. The separation of urinary estrogens not only gives information of physiological value that could not be gained by determining the total estrogenic potency but also higher values for 'total' estrogen than are acquired by the bioassay of crude extracts, apparently because of the removal, in the process of separation and purification, of substances which inhibit the action of estrogens. The use of phenolsulphonic acid for the colorimetric assay of estrogens from urines of pregnancy has been found unreliable, due to the presence, in many specimens, of interfering substances which react with this color reagent and which we have been unable to remove without loss of estrogens. The concentration of these interfering substances varies tremendously, both qualitatively and quantitatively, with different urines, making any correction for overestimation impossible.

STEPHENS, D. J. Suppression of lactation in acromegaly during estrogenic therapy. *To appear in this Journal.*

A patient with acromegaly is described, in whom persistent secretion from both breasts, macroscopically and microscopically characteristic of milk had become established. During estrogenic therapy, there was suppression of lactation, associated with striking subjective clinical improvement, lowering of an elevated blood pressure, and slight increase in glucose tolerance. There was no change in the general clinical appearance or in the B.M.R. during the administration of estrogenic substance.

ADRENALS

STAHL, J., D. KUHLMANN AND M. URBAN. Note on the mechanism of kidney insufficiency in experimental adrenal insufficiency (A propos du mécanisme de l'insuffisance rénale au cours de l'insuffisance surrénalienne expérimentale). *Compt. rend. Soc. de Biol.* 127: 1286. 1938.

The urine output of adrenalectomized dogs was studied. The volume excreted seemed to depend on the blood volume and not on any specific action of the cortical hormone on the kidney itself.—J. C. D.

THORN, G. W., L. L. ENGEL AND H. EISENBERG. Treatment of Adrenal Insufficiency by Means of Subcutaneous Implants of Pellets of Desoxy-corticosterone Acetate (A Synthetic Adrenal Cortical Hormone). *Johns Hopkins Hosp.* 64: 155. 1939.

Bilaterally adrenalectomized dogs, fed a constant diet of low Na and Cl content, were maintained in excellent condition, by means of the subcutaneous implantation of pellets of crystalline desoxy-corticosterone acetate. Removal of the pellets was followed by increased renal excretion of Na and Cl, weight loss, hemoconcentration, rise in N.P.N. of the blood, K retention and a decrease in the plasma concentration of Na and Cl. Reimplantation of the pellets resulted in a prompt and striking decrease in the renal excretion of Na and Cl, an increased K excretion, a restoration of plasma electrolytes to normal, improved appetite and weight gain. The quantity of hormone provided by pellets was determined by removing the pellets and weighing them. Experiments indicate that not only is the necessity for daily injections of hormone obviated but also a considerable saving in the hormone requirement of adrenalectomized dogs may be effected by the use of this technic.—Authors' Abstract.

WAGNER, B. Management of hyperemesis gravidarum with adrenal cortex hormone, a study of endocrine conditioned metabolic disturbances as causes of hyperemesis gravidarum (Die Behandlung der Hyperemesis grav. mit Nebennierenrindenhormon, ein Beitrag zur Frage der endokrin bedingten Stoffwechselstörung als Ursache der Hyperemesis grav.). *Zentrlbl. f. Gynäk.* 63: 432. 1939.

The AP fat and carbohydrate metabolism hormones are said to be increased in blood and urine of women with hyperemesis, and adrenal cortex therapy is said to reduce this excess. The involvement of these hormones in hepatic disease seems probable. Vitamins B_1 and C are also involved. The mechanisms are not clear. On this basis the author tries the use of pancortex, which represents in each cc. 50 g. cortex and 50 mg. ascorbic acid. Doses of less than 5 cc. seemed useless. In a series of 14 cases most of them in the first trimester, with severe and continued emesis, where commoner methods had failed, adrenal cortex and glucose were given intravenously, one or more times daily. Doses were from 5 to 20 cc., commonly 10 cc. Results were evident in 1 to 2 days, and treatment was seldom carried out for more than 4 days. There is no mention of any treatment after the emesis was brought under control. All cases went to term. In some threatened abortion was treated by use of progestin. No explanation is offered for the success followed by no further therapy. Recurrences occurred only twice.—E. L. S.

WILLSON, D. M., AND F. W. SUNDERMAN. Studies in serum electrolytes. XII. The effect of water restriction in a patient with Addison's disease receiving sodium chloride. *J. Clin. Investigation* 18: 35. 1939.

A patient with chronic tuberculous Addison's disease was maintained with a constant intake of NaCl and bicarbonate. Water intake was varied, and Na and Cl output studied. Restriction to the point of dehydration provoked a collapse very similar to a typical crisis, which was relieved by water administration. The serum Na concentration rose above normal levels during the crisis. The blood volume was abnormally low at this time. Water deprivation depressed Na and Cl excretion more than in the normal control who undertook all the routines for comparison. Forcing water intake had little effect, but appears to have caused a greater loss of salt in the patient than in the control. The serum salt and water content were not affected by the forced water intake.—E. L. S.

ZECKWER, I. T. The effect of combined thyroidectomy and gonadectomy on compensatory adrenal hypertrophy in unilaterally adrenalectomized rats. *Am. J. Physiol.* 123: 266. 1938.

The thyroids, gonads or both were removed from large groups of rats at 5 weeks of age; in some of these groups the left adrenal was removed at the same time or up to 4 weeks later. At 16 weeks of age the animals were killed and the adrenals and pituitaries observed. The removal of the thyroids or gonads or of both caused an increase in adrenal weights in male rats, but no significant change for those of the females. The compensatory hypertrophy of the intact adrenal following removal of the other adrenal was not affected by either thyroidectomy, or gonadectomy or by the 2 operations combined. It is therefore concluded that adrenal cortical growth is not controlled by the 'castration cells' nor by the 'thyroidectomy cells' of the pituitary, which arise after gonadectomy and thyroidectomy respectively. Since thyroidectomy also causes a loss of nearly all acidophiles, and by elimination of the non-secretory chromaphobes, it is believed that adrenal cortical growth is controlled by a basophilic cell that is not a 'castration cell' or a 'thyroidectomy cell'.—S. L. Cohen.

ENDOCRINE GENERAL

AGDUHR, E. Internal secretion and resistance to injurious factors. *Acta. Med. Scandinav.* 99: 387. 1939.

Various toxic substances, such as irradiated ergosterol, arsenic acid, magnesium sulfate, copper sulfate, alcohol, medinal, rat-typhus culture and diphtheria toxin, were administered to several thousand white mice, rats and rabbits. The effects of segregating the animals and of permitting them to mate were studied. It was found that sexual intercourse increased the resistance to the toxic substances in most cases. In the case of diphtheria toxin the animals that were prevented from mating until 90 days showed a higher resistance than those allowed to mate at 30 days. Certain sex differences also appeared, thus the females were more resistant to irradiated ergosterol, alcohol, and medinal but less so to typhus. The administration of estrone, testosterone and anterior pituitary hormones was also found to produce an antitoxin effect. These differences in resistance are considered to be due to hormonal influence and may explain in part the increased duration of life seen in human married couples.—J. M. L.

G. ÅGREN. On the preparation of cholecystokinin. *Skandinav. Arch. f. Physiol.* 81: 234. 1939.

A method is described for the preparation of nighly active cholecystokinin. By repeated electrophoresis at pH 4.25 and 6.60 a product was obtained which was free of secretin and vaso-dilatin and which in doses as small as 2 mg. caused contraction of the isolated guinea-pig's gall bladder.—A. G.

BLOTNER, H. Late results following the use of insulin in one hundred cases of malnutrition. *New England J. Med.* 218: 371. 1938.

The injection of 10 units of insulin 3 times a day before meals was used in 54 women and 46 men for malnutrition of non-diabetic origin. The treatment was taken for periods of 2 weeks to 7 months with an average duration of 6 weeks and an average gain in weight of 12.4 lb. The immediate effects were a gain in weight, a better appetite and an improvement in general condition. The majority of the patients maintained the added weight and improved general condition for as long as 6 years after discontinuing the insulin and one became definitely obese. The sugar tolerance decreased in some patients during treatment but returned to normal afterwards.—J. M. L.

ENGELHES, H. Sympathico-endocrine insufficiency. A hitherto undescribed syndrome. *Acta. Med. Scandinav.* 96: 231. 1938.

A description was given of 2 patients who presented similar symptoms of a morbid state not previously reported. In the initial stage there was marked acrocyanosis, later there was complete loss of adipose tissue, polyneuritis, edema, trophic ulcers and endocrine disturbances mostly of a suprarenal nature. In one case autopsy findings revealed no striking changes in the endocrine glands but there were

slight changes in the subthalamus. The symptoms were explained on the basis of a disturbance of the sympathetic innervation, with the most probable primary lesion occurring in the sympathetic basal ganglia in the mid-brain.—J. M. L.

Fuentes, M. Case of medulloblastoma associated with the adiposogenital syndrome. Clinical note. *Arch. neurol. y psiquiat de Mexico* 1: 296. 1938.

Fuentes reports the case of a boy aged 11 years who, 6 months after a fall on the head, experienced severe headaches, failing vision, vomiting, polyphagia, polydipsia and increase in weight. In the following 2 years the size of the head increased notably: blindness became complete; headache, vomiting and dizziness continued, and somnolence, disequilibrium and mental retardation, with incoherent speech and a tendency to confabulation, developed. Physical examination revealed a typical adiposogenital syndrome with testicular involution. Neurologic examination showed no definite localizing signs. Lumbar puncture revealed marked increase in pressure. A ventriculogram showed enormous dilatation of the lateral ventricles. Exploration with a right frontoparietal approach, was performed, but the tumor of the cerebellum, occupying the roof of the fourth ventricle, extending through the aqueduct and spreading to the internal walls of both lateral ventricles, the floor of the third ventricle and the pia over the vermis and cerebellar lobules. The tumor was diagnosed as a medulloblastoma or isomorphous glioblastoma. The author believes that the adiposogenital syndrome was produced by the tumor, although the hypophysis was normal.—Abstract from *Arch. Neurol. & Psychiat.*

Hemmingsen, A. M., A. Nielson and A. L. Nielsen. The effect of insulin on adrenalectomized rats and mice. *Acta. Med. Scandinav. Supplement* 90: 105. 1938.

Adrenalectomized rats and mice maintained on a high salt diet serve as very sensitive test animals for insulin. The mice react by convulsions but the rats by paralyses of the limbs. The dosage necessary to cause reactions in the rat is 10 to 20 times that required for the mouse. The normal rat responds to 0.2 I.U. per 100 gm. of body weight whereas the adrenalectomized rat reacts to 1/10 this amount, 0.02 I.U. With mice 0.002 I.U. can be detected.—J. M. L.

Kjems, H., and T. Bjering. A method for determining diabetogenous hormone in urine. *Acta. Med. Scandinav.* 99: 492. 1939.

The active diabetogenic hormone is precipitated from the urine by $ZnSO_4$ and $K_4Fe(CN)_6$ and the sediment washed with acetone. It is then taken up in ammonia and reprecipitated with acetone and saturated NaCl solution and finally dissolved in water. The material is assayed on fasting rabbits by subcutaneous injection and determination of the blood sugar.—J. M. L.

Page, I. H. The effect of bilateral adrenalectomy on arterial blood pressure of dogs with experimental hypertension. *Am. J. Physiol.* 122: 352. 1938.

The part played by the adrenals, gonads and hypophysis in the hypertension produced in 24 dogs by placing clamps on the renal arteries was studied. While unilateral adrenalectomy had no effect, bilateral adrenalectomy caused the hypertension to be rapidly abolished. This fall in blood pressure was much less marked and never complete if salt plus cortical extract therapy were instituted immediately after adrenalectomy. No hypertension was produced by placing the renal clamps in previously adrenalectomized animals. An attempt to link more closely the hypertension with the adrenal cortex by showing the persistence of hypertension after the removal of one adrenal and demedullation of the other was not completely successful in that subsequent histological section showed incomplete removal of the medulla. Neither the ovaries nor testes were found essential for the maintenance of the hypertension. Previous hypophysectomy and later removal of the testes in one animal were found to have no significant effect on the experimentally produced hypertension; adrenalectomy in this animal with or without cortical extract plus salt therapy had the usual effects. The author believes that the endocrine glands in these animals are concerned chiefly with maintenance of the body in such a state that it can respond to the renal artery constriction by the development of arterial hypertension.—S. L. Cohen.

Rudy, A., and M. M. Cohen. The oral aspects of diabetes mellitus. *New England J. of Med.* 219: 503. 1938.

A study of the dental condition of 403 diabetic patients was made. Very few changes were found in children. In adults frequent heavy tartar deposits usually supragingivally placed, with marked loosening of the teeth and hypertrophied, inflamed and bleeding of the gingiva were noted especially in patients in whom the diabetes was not adequately treated. The diabetic condition must be controlled before local dental treatment is instituted.—J. M. L.

TOUW, J. F., AND R. K. W. KNIPERS. The treatment of affections of the joints with progestin. *Acta. Med. Scandinav.* 96: 501. 1938.

Because of the improvement sometimes observed in women with arthritis during pregnancy, 10 u of progestin daily for 11 days was administered and resulted in marked improvement in 3 patients with arthritis. One patient remained free from complaints for 6 months and another for 4 months. Pregnyl (theelin?) in amounts up to 1000 u daily was without effect.—J. M. L.

GONADS

ANDERSON, E. L., AND G. F. MARRIAN. The identification of equol as 7-hydroxy-3 (4^1-hydroxyphenyl) chroman, and the synthesis of racemic equol methyl ether. *J. Biol. Chem.* 127: 649. 1939.

Equol, $C_{15}H_{14}O_2$, was shown to be 7-hydroxy-3(4^1-hydroxyphenyl) chroman as the oxidation products of its methyl ether was found to be identical with synthetic 7, 4^1-dimethoxy-2, 3-dihydroisoflavone. The technique for the preparation of the synthetic equol methyl ether was described.—J. M. L.

BISHOP, P. M. F., M. BOYCOTT AND S. ZUCKERMAN. The estrogenic properties of stilboestrol (diethylstilboestrol). *Lancet* 236: 5. 1939.

The synthetic hormone stilboestrol isolated in 1938, was administered to 46 women in various stages of ovarian insufficiency, and to 6 monkeys. It was given both by mouth in tablets containing 0.1 mg., 1 mg., 5 mg., and by intramuscular injections containing 1 mg. and 5 mg. of the active principle per cc. of solution. The action of stilboestrol simulated that of the natural estrogens. It produced an intermenstrual type of endometrium both in the human female and in the monkey; growth of the hypoplastic uterus; in amenorrhea it provoked bleeding of the estrin-withdrawal type; it relieved the pain of dysmenorrhea; relieved the symptoms of the menopause, restoring the appearance of the vulva and vagina when they become atrophic as a result of the estrogenic deficiency of the climateric. Painful swelling of the breasts was produced and in some instances proliferation and activation of the epithelium of the mammary glands were noted. However in 3 of the 46 women, toxic effects such as nausea, headache and vomiting appeared, apparently due to the relatively complete absorption of stilboestrol when given by the oral route.—L. B. S.

BOLAND, R. Use of testosterone propionate in prostatic hypertrophy. *South. M. J.* 32: 154. 1939.

Thirty-five cases of uncomplicated prostatic hypertrophy showed clinical improvement following testosterone injections. In 7 of these cases, biopsies were taken before and after treatment. Two of these are reported in detail with photomicrographs which show the marked preponderance of stroma over glandular tissue before treatment and the reversal of this relationship following the injections and associated with the clinical improvement.—J. C. D.

COLVIN, E. D., AND R. A. BARTHOLOMEW. Behavior of the basal metabolism in the course of developing toxemia of pregnancy: correlation with cholesterol, placental infarcts and retinal examination. *Am. J. Obst. & Gynec.* 37: 584. 1939.

The authors present data on a series of primipara in the later 'teens' indicating the high frequency of toxemias at this age. The occurrence of the toxic states was higher in those patients with an initial basal metabolism below normal (?standards used), and with a higher blood cholesterol. With development of toxemia the basal metabolism rose markedly. Their concept is that low basal metabolism and the associated hypercholesterolemia predispose to injury of the fatal placental vessels by trauma from fetal movements, which leads to infarcts on the fetal side of the placenta. The absorption of toxic materials (?amines) into the maternal circulation causes a toxemia.—E. L. S.

CORNER, G. W. Quantitative studies of experimental menstruation-like bleeding due to hormone deprivation. *Am. J. Physiol.* 124: 1. 1938.

The relationship between estrin and progesterone in producing hormone-deprivation uterine bleeding was studied in 11 castrated monkeys. Discontinuance of the progesterone injections after a 10-day period of administration of suitable amounts of both estrone and progesterone was followed by uterine bleeding, even though the animals were still receiving sufficient estrone to prevent estrin-deprivation bleeding. From determinations of the minimal progesterone and estrin doses required to prevent the uterine bleeding, it is calculated that the above effect can not be due merely to a lowering of the total hormone level by the discontinuance of progesterone. The author believes, rather, that progesterone in some way suppresses the action of estrin upon the endometrium, while itself preventing uterine bleeding; subsequent withdrawal of the progesterone would then deprive the animal of both hormones. The significance of this interpretation in explaining normal menstrual bleeding is discussed.—S. L. Cohen.

Cuatrecasas, J. and A. A. Bruno. Metabolism of cholalic acid. (Contribución al estudio del metabolismo del ácido colálico.) *Rev. sud-americana endocrinol.* 22: 86. 1939.

The possible origin of sex hormones from biliary acids was investigated. Rabbits were treated for varying periods with pregnancy urine extracts (prolan) and the quantity of bile in the vesicle as well as its cholalic acid content determined. It is claimed that the quantity of bile and even more the cholalic acid decrease progressively with intensity of treatment. The figures submitted, however, do not support this claim. Liver pulp was mixed with ox bile and incubated for 24 hours. Injected into immature rabbits, it produced a strong estrogenic effect within 48 hours. Rabbits treated with small doses of prolan showed initially an increase of blood cholesterol, followed by a considerable decrease. Large doses produced an immediate decrease. Cholalic acid was normally not found in the rabbits blood; after prolan injection small quantities appeared.—A. E. M.

Dantchakoff, V. The effects of male hormone on a young female guinea pig whose treatment began prenatally (sex inversion) (Sur les effets de l'hormone male dans un jeune cobaye femelle traite depuis un stade embryonnaire (inversions sexuelles). *Compt. rend. Soc. de Biol.* 127: 1255. 1938.

Three weeks after insemination the embryo was given an injection of testosterone. Postnatally the animal received testosterone in various forms. It looked and acted like a male. It was killed at the age of 75 days. Both male and female accessory internal organs were present; the external genitalia were male and the gonads both female.—J. C. D.

Day, R. V. Male sex hormone therapy. *J. Urology* 41: 210. 1939.

This report is based on 126 male and 27 female patients. Adequate urological examination is essential. Hormone therapy should not be regarded as a substitute for surgery in cases of major prostatic obstruction. "In grade 1 and grade 2 benign hypertrophy, patients are afforded marked relief following use of male sex hormone, especially testosterone propionate; even the distress of large hypertrophies is sometimes palliated for a considerable period of time." Continued intensive treatment of patients under the prostatic age with male sex hormone is to be avoided.—J. C. D.

Deanesly, R., and A. S. Parkes. Further experiments on the administration of hormones by the subcutaneous implantation of tablets. *Lancet* 235: 606. 1938.

The weight of the estrone and estradiol tablets were between 5 to 20 mg. and 80 to 100 mg. Testosterone propionate (Ciba) weighed 100 mg. The tablets were inserted by making a slit in the skin (under ether anesthesia) and pushing the tablet into the subcutaneous tissue. The tablets could be easily recovered by a second operation. In the rat, the rate of absorption of estrone from tablets is between 2½ to 10% per month, testosterone propionate 15% per month, and that of testosterone 25% per month. It is suggested that this technic might be useful when a long continuous and steady effect is required.—L. B. S.

Del Castillo, E. B., and A. Pinto. Seasonal variations in the seminal vesicles of the white rat (Variaciones estacionales del peso de las vesículas seminales de la rata blanca). *Rev. Soc. Argent. de Biol.* 14: 462. 1938.

The weight of the seminal vesicles was determined in 294 white rats weighing between 140 and 240 gm. They were killed in every month of the year during 2 years. In autumn and winter the weight decreased reaching its lowest in April-June, to increase again in spring reaching a maximum in summer (November-February). In summer (November) the seminal vesicles were distended by an albuminous fluid; the wall consisted of a thin coat of smooth muscle and an epithelial layer which formed few villae. In April the muscular coat was thick, the epithelium formed numerous villae and there was little or no fluid in the cavity.—J. T. L.

Desclin, L. Note on the androgenic activity of the ovary (A propos de l'activité androgénique de l'ovaire). *Compt. rend. Soc. de Biol.* 128: 557. 1938.

In castrated white rats carrying ovarian grafts, the prostate gland shows, 3 months after grafting, signs of stimulation as though acted on by an androgenic substance. The lutein cells may be the source of this substance. To test this, 10 rats were castrated and injected with luteinizing hormone. Twenty others were castrated and then ovarian grafts were implanted. Ten of these received luteinizing hormone also. All were killed 25 days after castration. The grafts of rats receiving injections showed large numbers of lutein cells. The seminal vesicles of the grafted animals showed enlargement due to the stimulating effect of folliculin from the ovary on the muscle. The epithelium of neither the vesicle nor the prostate showed any signs of stimulation but remained in the same condition as that of the non-grafted castrated controls.—J. C. D.